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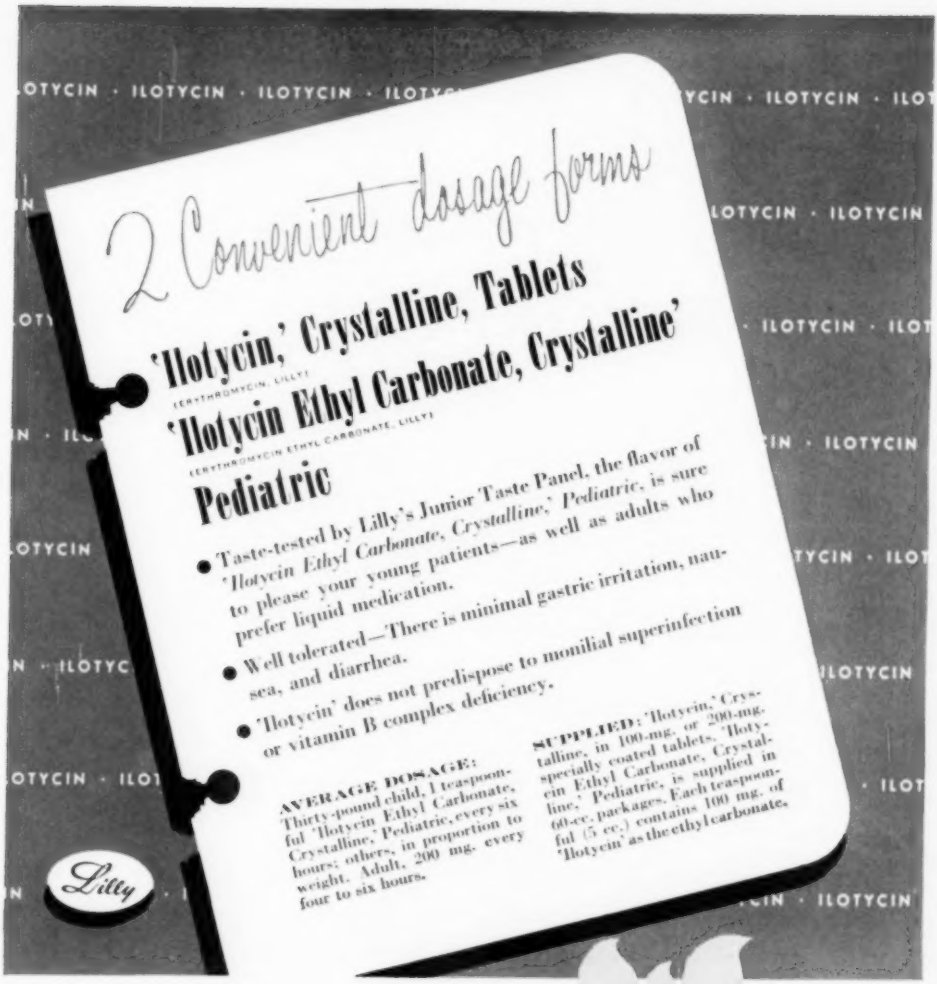
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3. Bishop, P. A., and Lindskog, G. E.: Lung Abscess, in Pillmore, G. U.: Clinical Radiology, Philadelphia, F. A. Davis Company, 1950, vol. 1, p. 339.

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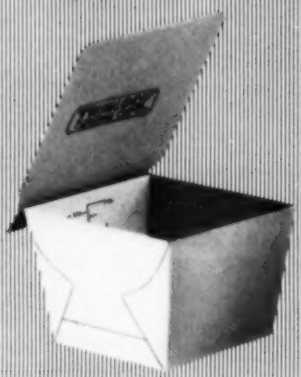

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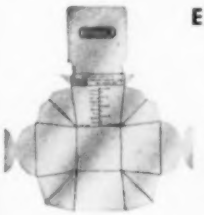

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THE AMERICAN REVIEW
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SCALENE NODE BIOPSY[1]

LAWRENCE M. SHEFTS,[2] ARTHUR A. TERRILL,[3] AND HERBERT SWINDELL[4]

(Received for publication May 19, 1953)

INTRODUCTION

In 1949, Daniels (1) reported the use of scalene lymph nodes in the diagnosis of certain intrathoracic diseases. He reported positive findings in scalene nodes in two cases of Boeck's sarcoid, two carcinomas of the lung, and one case of silicosis with demonstration of the silica particles by means of a Nicol prism. Storey and Reynolds (2) recently reported positive findings in scalene nodes in three cases of Hodgkin's disease, one carcinoma of the lung, and one Boeck's sarcoid. Single cases of successful diagnosis by use of the procedure were reported by Weiss, Campbell, and Budenz (3) (Boeck's sarcoid) and by Johnson and MacCurdy (4) (histoplasmosis).

In 1949, immediately following Daniels' ingenious observation, the present writers were stimulated to use this type of biopsy in a group of patients with as many different conditions as were available. The present report adds nothing fundamentally new to Daniels' original contribution but is intended as an additional report which may be of value in estimating the *relative* value of this type of biopsy in a variety of intrathoracic conditions.

The basis of this type of biopsy lies in the fact that the lymph nodes anterior to the scalenus anticus muscle are connected to the mediastinal nodes. Thus, pulmonary parenchymal lesions which might involve mediastinal nodes are reflected in the scalene nodes. The same is true of systemic conditions directly involving the mediastinal nodes. In a very real sense, this simple scalene node biopsy is a biopsy of the mediastinal nodes without the necessity of entering the thorax. The percentage of positive findings in the nodes varies with the pathologic conditions and the accuracy of the dissections.

[1] Presented before the Medical Session, as part of the symposium on *Surgery in the Treatment of Tuberculosis*, at the 49th annual meeting of the National Tuberculosis Association, Los Angeles, California, May 19, 1953.

[2] Civilian Consultant in Thoracic Surgery: Brooke Army Hospital, Fort Sam Houston, Texas; Thoracic Surgery Center, U. S. Air Force Hospital, Randolph Air Force Base, Texas; Veterans Administration Hospital, Kerrville, Texas; and Thoracic Surgery Service, Robert B. Green Memorial Hospital, San Antonio, Texas.

[3] Thoracic Surgery Section, Brooke Army Hospital, Fort Sam Houston, Texas.

[4] Thoracic Surgery Center, U. S. Air Force Hospital, Randolph Air Force Base, Texas.

Technique

The operation is usually done under local anesthesia. Careful gentle retraction will permit it to be done when the anesthesia is limited to simple skin infiltration. The position of the patient, incision, and approach are similar to the standard approach for the phrenic nerve and have been adequately described by Daniels (1). When the fascia over the scalenus anticus muscle is opened, a fat pad pops out and it is this pad which contains the desirable nodes. The pad is grasped and the dissection carried inferiorly and medially along the scalenus anticus muscle, removing as large an amount of the fat pad as possible. The operator should dissect the nodes cleanly from the fat pad before sending all of the material to the pathologist. The present writers have purposely refrained from going down into the mediastinum, in an effort to make the operation as simple and as safe as possible. Hence, in all of the cases of the present series, the surgical procedure was confined to the above-described operative area.

The transverse cervical vessels are often seen in the inferior portion of the field, and it is well to retract them at the start of the procedure. Occasionally these vessels are not seen and are inadvertently involved in the dissection as the fat pad is retracted. It is important to carry the dissection as far as possible medially and inferiorly unless an obvious grossly pathologic node is encountered.

The thoracic ducts are not searched for; but careful dissection, visualization, and clamping and ligation before transection of tissue will reduce the percentage of torn and transected ducts. It is the present policy to ligate all tissue before transection. The right duct has been visualized once; and the left as it arches up in the base of the neck, numerous times. Some of the branches coming down from the neck on the left have been visualized and, in spite of visualization, have been torn near the arch of the duct. Needless to say, these must be ligated.

The majority of the patients in the present series were hospital patients, but approximately 20 per cent of the total were treated as "release" patients. This means that the patients were taken to the operating room, operated upon, and returned home without the necessity of hospitalization. The present writers are opposed to the use of scalene node biopsy as an office procedure.

The operations were done by a total of eight surgeons. Six were members of a residency training program, being either residents or assistant residents. Although the operation is simple, it should not be done by interns.

The accuracy of dissection very materially affects the percentage of positive nodes. In two instances, nodes were originally reported as showing no abnormalities. As a result of further experience, it was believed that the nodes should have revealed positive findings in these cases, and reoperation on the same side resulted in a positive result in both cases (Boeck's sarcoid). Experience in dissecting out the fat pad and nodes is important, and preliminary cadaver dissection will enable one to do the operation far more efficiently. It is important to obtain the proper scalene nodes for, in 3 cases in the present series, nodes just superficial to the deep fascia were normal, while those immediately deep to the fascia revealed positive findings. It is of interest to note that the percentage of positive findings in the biopsy material increased in direct proportion to the total number done by the individual surgeon. This was the constant experience of all of the eight surgeons.

In 7 instances, nodes were reported as not being found. In only 2 cases could

permission for reoperation be obtained, and in both cases nodes were found at reoperation. The 5 patients not reoperated upon are not included in this series as it is believed that the failure to find the nodes was the result of inexperience.

Material

The total material of the present study consisted of 205 scalene node dissections, including 17 bilateral dissections, in 187 patients.

The group consisted of patients seen at the medical and surgical chest conferences at Brooke Army Hospital, Fort Sam Houston, Texas; and at the Thoracic Surgery Center, U. S. Air Force Hospital, Randolph Air Force Base, Texas; private patients; patients from the Veterans Administration Hospital, Kerrville, Texas; and the patients from the Thoracic Surgery Service of a county hospital (Robert B. Green Memorial Hospital, San Antonio, Texas).

Of the total of 187 patients, 154 (82.4 per cent) were white and 33 (17.6 per cent) were nonwhite; 170 (90.9 per cent) were male and 17 (9.1 per cent) were female.

The average age of the group was 36.4 years. The youngest patient was 17 years old and the oldest was 74. As many of the patients were young soldiers or inductees, it is of interest to point out that 37.5 per cent were 25 years of age or under.

Routine Tissue Study

All nodes were examined by hematoxylin and eosin stains. Ziehl Neelsen stains were used when indicated. Seabury (5) pointed out the similar microscopic appearance of fungal infections and Boeck's sarcoid. For this reason, Hotchkiss-McManus stains (6) were done in instances of nodes positive for Boeck's sarcoid. Unfortunately, it was only in the latter part of the series that routine cultures were made from the nodes for both tuberculosis and fungi. This is an extremely important part of the examination and it is believed, in looking back over some of the early cases, that the chance of a proper diagnosis has almost certainly been missed because of failure to obtain the cultures.

OBSERVATIONS

Complications

Complications were mainly limited to involvement of the thoracic ducts. The left thoracic duct was torn once; and the right twice. This makes three complications in 205 dissections (1.4 per cent). In none of the instances were the operators aware of this complication at the time of surgery. From the amount of drainage through the skin incision postoperatively, the involvement of the main duct on the left was unquestionable. In this instance, a case of bronchogenic carcinoma, the node was uninvolved and, following a left total pneumonectomy, the drainage ceased. Although at resection the thoracic duct was not visualized, everything possible was ligated in the mediastinum. In two instances of right duct involvement, it is possible that only branches were involved because of the small amount of drainage and the fact that aspiration and pressure dressings sufficed. In order to minimize possible involvement of the thoracic duct or the branches,

leaving the fat pad in place and dissecting out the nodes *in situ* may be worth while, but it makes finding the nodes more difficult.

In one instance a temporary right Horner's syndrome was noted.

Results

In order to more accurately estimate the value of scalene node biopsy, *only those cases not otherwise diagnosed* by other methods are considered. Thus, when a diagnosis was proved microscopically by sputum studies, bronchoscopic biopsy, bronchial secretions, sternal puncture, liver puncture, or other anatomically situated lymph nodes, that case was excluded from the present series. In 187 patients, 205 scalene node dissections (17 bilateral) were done.

TABLE 1

HISTOPATHOLOGIC DIAGNOSIS OF NODES REMOVED BY SCALENE NODE BIOPSY IN 17 PATIENTS (PILOT SERIES)

Boeck's sarcoid		8 patients
Bronchogenic carcinoma		6 patients
Undifferentiated	4	
Adenocarcinoma	2	
Hodgkin's disease		1 patient
Tuberculosis		1 patient
Hypernephroma		1 patient

TABLE 2

SCALENE NODE BIOPSY. INCIDENCE OF ABNORMAL FINDINGS AMONG 187 PATIENTS WITH PREVIOUSLY UNDIAGNOSED INTRATHORACIC DISEASE

Total series, 187 patients	205 biopsies (17 bilateral)
Number with abnormal scalene nodes	67 patients
Percentage of positive findings	35.8

In 1949, a *pilot series* (private cases) was begun to determine the value of the procedure for further extended study. Twenty-five biopsies, including 2 bilateral, were performed in 23 patients. In 17 of these patients (73.9 per cent), the nodes were positive for a variety of pathologic conditions. The pathologic conditions found are listed in table 1. They were Boeck's sarcoid in 8 patients; bronchogenic carcinoma in 6 patients (4 undifferentiated, 2 adenocarcinoma); and one instance each of Hodgkin's disease, tuberculosis, and hypernephroma.

The above results were obviously sufficient reason to broaden the scope of the indications for the procedure. The present report, therefore, includes the results both in this initial series of 23 patients and in 164 additional patients.

In the entire series of 187 patients, 205 biopsies (including 17 bilateral biopsies) were done. In 67 of these patients (35.8 per cent), the biopsy material revealed positive evidence of the identity of the intrathoracic disease. The results obtained in this *total series* of 187 patients are presented in table 2.

The types of pathologic conditions found in the series of 187 patients are

shown in table 3. Boeck's sarcoid was present in 38 patients; bronchogenic carcinoma in 13 (9 undifferentiated, 4 adenocarcinoma); tuberculosis in 8 patients; lymphosarcoma in 2; Hodgkin's disease in 2; and histoplasmosis in one patient. In addition, there were 3 cases of metastases to the lung (by carcinoma of the pancreas, hypernephroma, and carcinoma of the cervix).

TABLE 3

SCALENE NODE BIOPSY. TYPES OF DISEASE REVEALED BY EXAMINATION OF TISSUE FROM 187 PATIENTS WITH PREVIOUSLY UNDIAGNOSED INTRATHORACIC DISEASE

Abnormal nodes		67 patients
Disease of nodes		
Boeck's sarcoid		38
Bronchogenic carcinoma		13
Undifferentiated	9	
Adenocarcinoma	4	
Tuberculosis		8
Lymphosarcoma		2
Hodgkin's disease		2
Histoplasmosis		1
Metastases of tumor to the lung		3
Carcinoma of pancreas	1	
Hypernephroma	1	
Carcinoma of cervix	1	

TABLE 4

INCIDENCE OF POSITIVE FINDINGS FROM SCALENE NODE BIOPSY ARRANGED ACCORDING TO SITE OF DISSECTION

	TOTAL BIOPSIES	NORMAL	ABNORMAL	PERCENTAGE ABNORMAL
Right				
Unilateral	119	76	43	
Bilateral	17	12	5	
Total	136		48	35.3
Left				
Unilateral	52	37	15	
Bilateral	17	11	6	
Total	69		21	30.4

The problem of which side of the neck should be explored for biopsy may, in part, be answered by the material presented in table 4. As may be seen, there is little difference statistically between the right and left scalene nodes in terms of the incidence of positive findings (35.3 per cent positive on the right as compared with 30.4 per cent positive on the left). The fact that there were 136 right-sided biopsies in contrast to only 69 on the left is explained by the fact that, in

instances of equal bilateral intrathoracic involvement, the right scalene node biopsy was selected as being the safer procedure. These were principally cases of suspected fungal infections, especially histoplasmosis. It is obvious, however, that usually and quite properly the scalene nodes on the same side as the principal intrathoracic involvement would be the ones chosen for biopsy. When there is no pulmonary lesion and the enlargement of the mediastinum is equal bilaterally, it is probable that the highest percentage of positive nodes would be found on the left.

In the 17 bilateral cases, there were 9 nodes which revealed positive findings. There were only 2 instances in which both right and left scalene nodes revealed

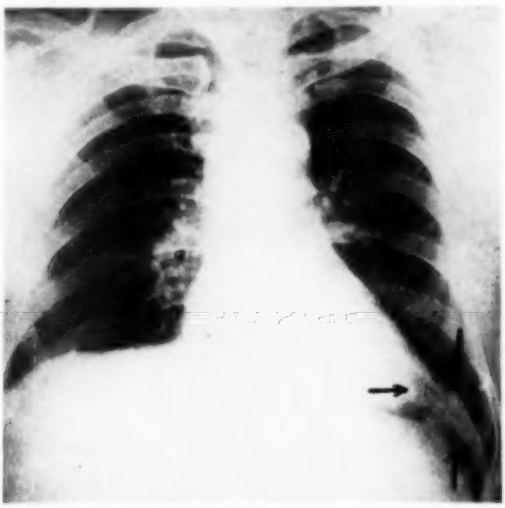

FIG. 1. *Case 1*. Boeck's sarcoid. Infiltration in left lower lobe as delineated by arrows.

positive findings: one, a case of undifferentiated bronchogenic carcinoma in the left lower lobe with a markedly enlarged mediastinum; and the other, a case of Boeck's sarcoid with an equally enlarged mediastinum. In the remaining 7 bilateral dissections, the biopsy material from 6 was positive for Boeck's sarcoid, and from one was positive for tuberculosis. In reviewing the roentgenograms of these patients, the node was positive, as expected, on the side of the most prominent extension of the mediastinal enlargement. However, if, in a given case in which a positive node is expected, the node is normal, it is advisable to dissect the other side. An example of the validity of this statement, as well as the unexpected results from a bilateral dissection, is as follows:

Case 1. The patient, A. S., was a 54-year-old white male. He had an attack of "influenza" in January and February, 1951, with pain on the left side radiating to the back. In April 1951, he began to have occasional small hemoptyses. A chest roentgenogram (figure 1) revealed a small area of infiltration in the left lower lobe adjacent to the left cardiac border

The enlargement of the mediastinum on this film was not noted on the original examination. Bronchoscopy in June, 1951, was completely negative for all studies, and a presumptive diagnosis of bronchogenic carcinoma was made. A *left* scalene node biopsy was done and the tissue was reported to be normal. The patient refused exploratory thoracotomy, and it was not until September, 1951, that he returned because of repeated hemoptyses. A

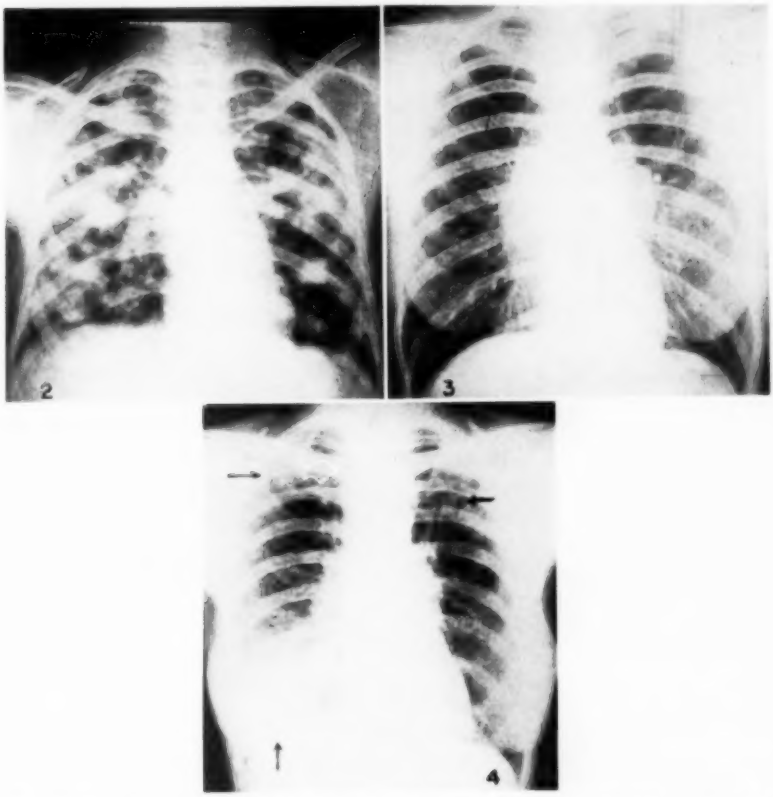

FIG. 2. Boeck's sarcoid. Marked bilateral pulmonary infiltration with hilar and mediastinal enlargement.

FIG. 3. Boeck's sarcoid. Bilateral hilar masses with no discernible pulmonary parenchymal involvement.

FIG. 4. Boeck's sarcoid. Variety of pulmonary infiltrations ranging from small areas of infiltration to lobar involvement, as delineated by the arrows.

right scalene node biopsy was done and the tissue was reported to be positive for Boeck's sarcoid. There had been no change in the roentgenographic findings in this interval of time. In spite of the positive microscopic diagnosis of Boeck's sarcoid, it was believed that thoracotomy was indicated because of the combination of hemoptysis in a 54-year-old patient with the above roentgenographic findings. (None of the other 37 patients with Boeck's sarcoid had hemoptysis.) Left thoracotomy was done on October 3, 1951, with a

wedge resection of the lesion in the left lower lobe. Immediate frozen section was diagnosed as Boeck's sarcoid and this was confirmed by later hematoxylin-eosin stain. The patient is still alive and has occasional small hemoptyses.

Boeck's Sarcoid

Boeck's sarcoid was by far the most common diagnosis in the series of 187 patients. As the roentgenogram of the chest was the first lead in arriving at the diagnosis, it is appropriate to review the roentgenographic findings.

> In the main, the roentgenographic findings may be divided into three groups. The first group (figure 2) consisted of patients with enlargement of hilar and mediastinal nodes and pulmonary involvement ranging from single or multiple areas of discrete opacities or lobar involvement to a fine lacelike network equally distributed throughout all parts of the lungs. The second group (figure 3) consisted of patients with large hilar masses of enlarged nodes with no or almost no discernible pulmonary parenchymal involvement. The third group (figure 4) consisted of patients without evident hilar enlargement, and the findings were limited to the varied pulmonary parenchymal involvement described in the first group.

It is believed to be certain, after the experience of following a number of these cases for a period ranging from one to four and one-half years, that the above described roentgenographic findings are merely different phases of the same process. In a given case of Boeck's sarcoid, it is mere chance which type of roentgenographic finding is noted at any particular time. Boeck's sarcoid, if followed long enough through its periods of exacerbation and remission, will present all of the variable findings above listed. It is sufficient to know the variety of roentgenographic manifestations so that the index of suspicion will be sufficiently acute to consider this condition and lead to performance of a scalene node biopsy.

These three groups of roentgenographic findings are arbitrary indeed, for it is believed that, in all cases in which the scalene nodes are positive, the mediastinal nodes are enlarged but may not be readily evident in a particular roentgenographic examination. In all of the scalene nodes positive for Boeck's sarcoid, the scalene nodes were enlarged, and it can be assumed that the same is true of the mediastinal nodes.

Bronchogenic Carcinoma

The value of scalene node biopsy in bronchogenic carcinoma is twofold: First, the procedure is of value in obtaining, not only a diagnosis, but also the exact microscopic type of the tumor. Second, in the instance of positive scalene nodes, the information that carcinoma has extended outside the immediate confines of the chest is readily apparent.

The 13 cases in which carcinomatous nodes were found were not microscopically diagnosed by any other diagnostic measure. Figures 5 through 9 reveal the roentgenographic findings which were typical of these cases. Scalene nodes examined in 24 nonconsecutive cases of bronchogenic carcinoma produced the

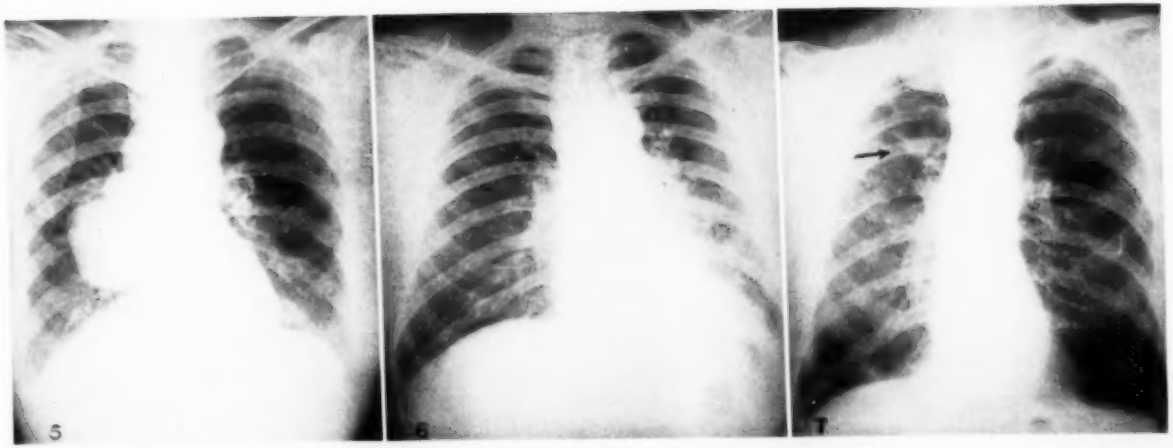

FIG. 5. Bronchogenic carcinoma. Large tumor mass in right lower lobe.

FIG. 6. Bronchogenic carcinoma. Partial atelectasis, left lower lobe. Enlargement of mediastinum at level of aortic arch. Scalene nodes on both sides contained carcinoma.

13 nodes positive for carcinoma. In the remaining 11 patients with uninvolved scalene nodes, 4 refused operation. These 4 patients are either still living or, if dead, no autopsy was done and, therefore, information concerning mediastinal node involvement is lacking. In the remaining 7 patients with uninvolved scalene nodes, all were operated upon and resections were performed in 6 of them. One of the 6 had carcinoma in the mediastinal nodes. The seventh patient had massive mediastinal node involvement so extensive as to make it technically inoperable.

Although this portion of the series is too small to be statistically significant, it should be pointed out that in only 2 of the 7 patients who had thoracotomies

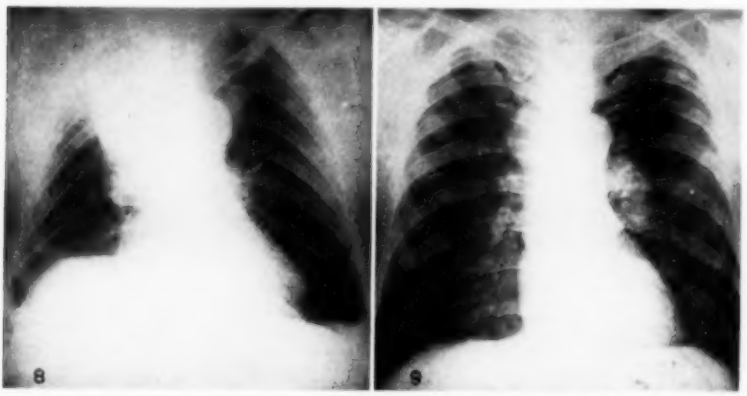

FIG. 7. Bronchogenic carcinoma. The arrow points to a very small infiltration in the right upper lobe.

FIG. 8. Bronchogenic carcinoma. Complete atelectasis, right upper lobe, and massive mediastinal involvement.

FIG. 9. Bronchogenic carcinoma in the left hilar area with mediastinal involvement. Left scalene node contained carcinoma.

were the scalene nodes uninvolved in the presence of carcinomatous involvement of the mediastinal nodes. These figures will have little significance until a much larger group of bronchogenic carcinoma is so investigated.

It is considered noteworthy that, of the 13 carcinomatous scalene nodes, the tumors in 9 were undifferentiated in type, and in 4 patients were adenocarcinoma. No squamous cell carcinomas were found in the nodes. In the 7 patients with uninvolved scalene nodes who had thoracotomies (2 patients had mediastinal involvement), all of the tumors were squamous cell carcinoma.

Tuberculosis

In the 8 cases of tuberculosis, the roentgenographic findings were simply those of mediastinal and/or hilar enlargement, there being no discernible parenchymal involvement. These patients had sputum examinations negative for tubercle bacilli (a minimum of 3 negative concentrates of twenty-four-hour

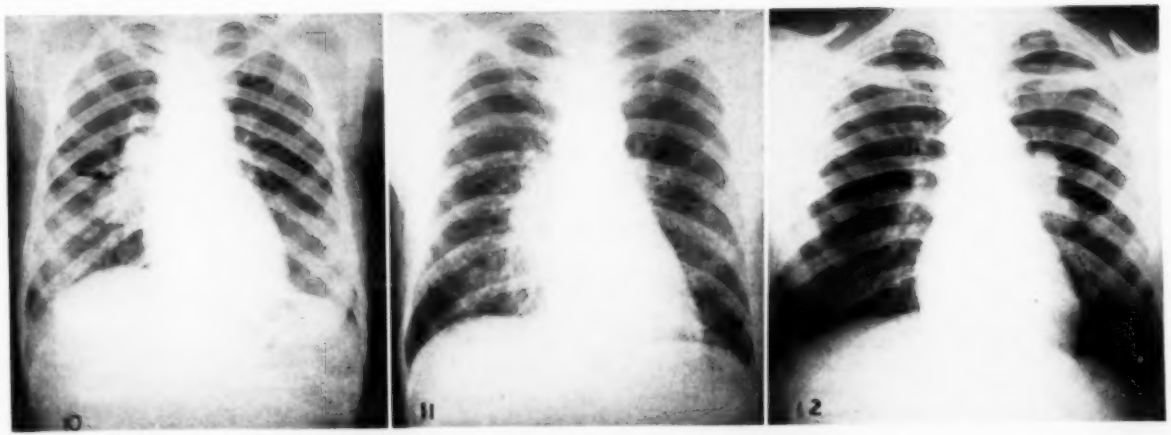

Fig. 10. The arrows point to areas of hilar and mediastinal node involvement by tuberculosis.
Fig. 11. Enlargement of the right hilar nodes by tuberculosis.
Fig. 12. Tuberculous involvement of the left hilar nodes extending up into the mediastinal nodes.

sputum collections and 3 negative cultures) and normal bronchoscopic findings. Figures 10, 11, and 12 are examples of such cases. In all of the cases in which the scalene nodes were positive for tuberculosis, the nodes were enlarged, grossly necrotic, and positive for tubercle bacilli both by tissue stain and by culture. Frequently the nodes were a conglomerate caseous mass invading the scalene fat pad. There can be little question that, considering the gross appearance of the nodes in these cases, the assumption was correct that the involvement of the mediastinal nodes was tuberculous. The important question which has arisen, however, is the significance of a scalene node positive for tuberculosis when there is neither a pulmonary lesion nor mediastinal enlargement on roentgenographic examination. As routine cultures of the nodes were made late in the present series, this question cannot be answered at present. In the opinion of the writers, the question can best be answered by study of a large number of scalene nodes obtained at autopsy on both tuberculous and nontuberculous patients. Needless to say, the scalene nodes in such a study should be compared with the mediastinal nodes in each autopsy. Such a study is now in progress. Knowledge of the percentage of patients with no history or findings of clinical tuberculosis who have tuberculous scalene nodes would be of importance in evaluating instances of undiagnosed intrathoracic disease associated with tuberculous scalene nodes. It is hoped that raising this question will stimulate further work on this subject.

Lymphomas

In the cases of lymphoma, as in the cases of bronchogenic carcinoma, the scalene node biopsies were the only positive method of diagnosis. As lymphomas occasionally may remain confined for a long time to one portion of the body (in these instances, the chest), it becomes important to arrive promptly at a proper diagnosis by as simple a method as possible. The roentgenograms of both cases of lymphosarcoma and of one of the 2 cases of Hodgkin's disease are presented in figures 13, 14, and 15. In these two diseases, the roentgenographic findings are often difficult to differentiate from those of metastatic malignancy. Hence, a microscopic diagnosis obtained by a scalene node biopsy may not only eliminate an unnecessary thoracotomy but also lead to the prompt use of presently accepted proper therapy.

Fungal Infections

The roentgenographic manifestations of fungal infections are protean, ranging from single small cavities to multiple large ones, from small areas of infiltration to large areas of bilateral involvement, and varied combinations of the above. Perhaps the incidence of a frequently shifting roentgenographic density in a patient relatively well should make one suspicious of a fungal infection. The typical multiple minute speckling of the lungs in histoplasmosis may be highly suggestive. Such a diagnosis was made by culture of the scalene node in one case (figure 16). Cultures are important, for the hematoxylin-eosin section is often difficult to interpret.

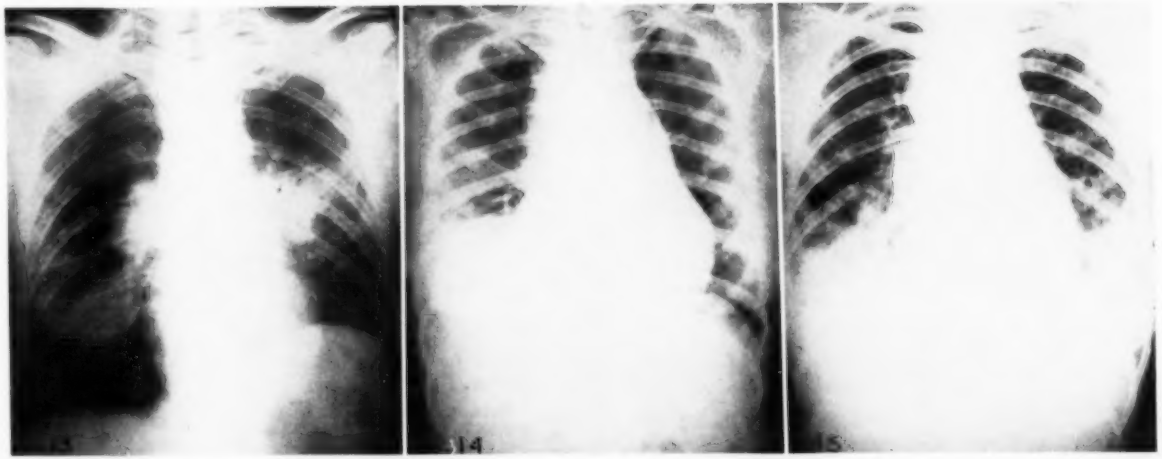

FIG. 13. Lymphosarcoma. Bilateral involvement of the hilar nodes with pulmonary parenchymal involvement on the left and mediastinal involvement.

FIG. 14. Lymphosarcoma. Massive mediastinal enlargement with right pleural effusion.

FIG. 15. Hodgkin's disease. Massive mediastinal enlargement and marked involvement of both lower lobes by conglomerate infiltrations.

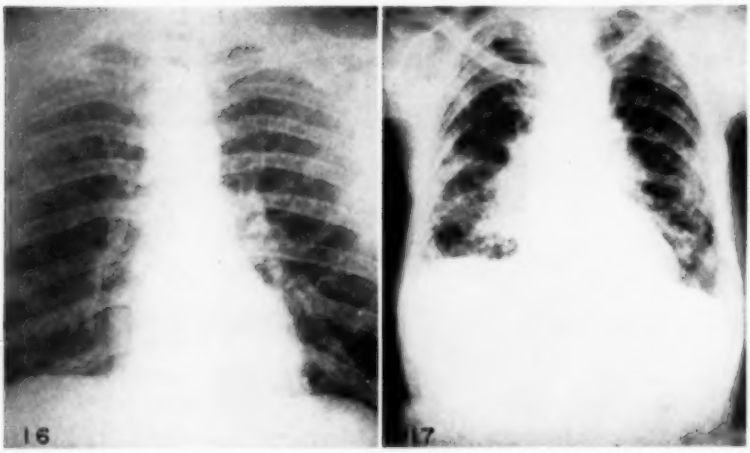

FIG. 16. Histoplasmosis. Speckling of both lung fields with minute areas of calcification and fibrosis.

FIG. 17. Blastomycosis. Bilateral infiltration involving the greater portions of both lungs.

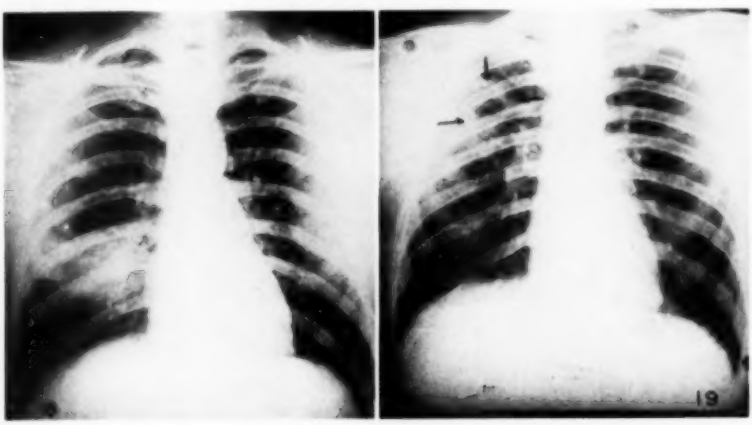

FIG. 18. Actinomycosis. Infiltration limited to the right middle lobe.

FIG. 19. Coccidioidal cavitation. The cavity in the right upper lobe is delineated by arrows.

Scalene nodes positive by culture for one case each of actinomycosis and coccidioidomycosis and 2 cases of blastomycosis (one North- and one South-American types) were obtained. These 4 cases (figures 17, 18, and 19) were not included in the series, for the organisms were previously obtained by smear or culture, or both, of the sputum or bronchoscopic washings. They are listed here merely to show that fungal infections of the lung are in some instances reflected in the scalene nodes.

Metastatic Pulmonary Lesions

Frequently, with metastatic pulmonary lesions, the patient's clinical history and physical findings yield no clue concerning the primary site. The microscopic type of metastatic carcinoma, as found in the readily available scalene nodes, may be of aid in tracking down the primary site. The following cases are examples of this type of situation.

Hypernephroma—Case 2. The patient, W. H. H., was a 46-year-old white male who developed acute pneumonitis in November, 1949. The course of the illness was protracted

FIG. 20. *Case 2.* Metastasis from hypernephroma. Bilateral massive pulmonary infiltrations.

over a three-month period. Following this illness, for approximately two months, the patient felt fairly well. He then began to note difficult breathing and began to have hemoptysis and pain in the chest. This was followed by a cough which became continuous and a weight loss of 50 pounds in one year. A roentgenogram of the chest revealed that the lower two-thirds of both lung fields were studded with large conglomerate masses (figure 20). *Right* scalene node biopsy, performed on December 20, 1950, yielded tissue containing typical hypernephroma cells. The correctness of this diagnosis was proved by autopsy.

Carcinoma of the pancreas—Case 3. The patient, J. G., was a 34-year-old white male captain in the Air Force. In the fall of 1949, he began to complain of intermittent right upper quadrant pain. This was not associated with type or time of meals and was accompanied by occasional nausea and vomiting. In the next two and one-half years the patient was in a number of Air Force hospitals and had numerous gastrointestinal studies, as well as gallbladder studies. In only one was there any reported positive finding and that consisted of

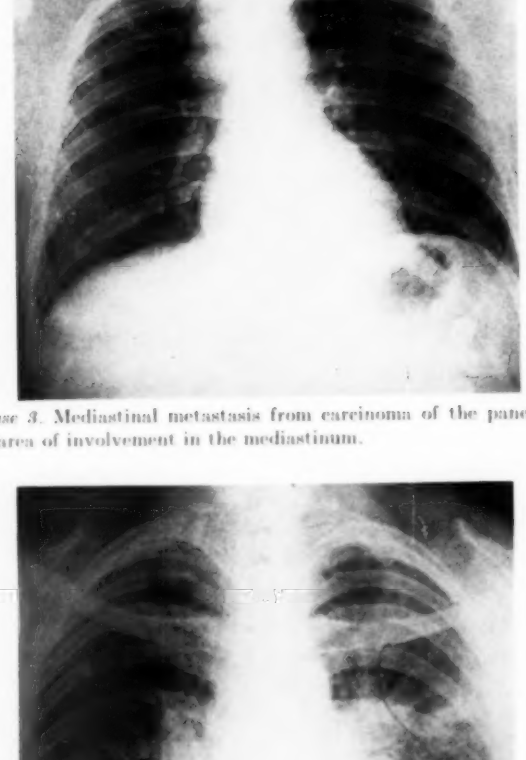

FIG. 21. *Case 3.* Mediastinal metastasis from carcinoma of the pancreas. The arrow points to the area of involvement in the mediastinum.

FIG. 22. *Case 4.* Metastasis from carcinoma of the cervix. Bilateral pulmonary infiltrations in the lower halves of both lung fields.

"deformity of the duodenal cap—no obvious ulcer." By June, 1952, he had developed more abdominal pain, and in August, 1952, he developed a grayish maculo-papular skin rash over the chin which was moderately pruritic. This lesion increased and involved the skin of the lower face, upper portion of the neck, axillary folds, umbilical region, and perineum. In

November, 1952, he developed a dry hacking cough and moderate pain in both chests. By December, 1952, shortness of breath became evident.

On admission to the Station Hospital, Randolph Air Force Base, Texas, January 16, 1953, the patient acknowledged a 25-pound weight loss since July, 1952. A chest roentgenogram (figure 21) revealed a mass in the mediastinum. Gastroscopy was negative. Studies of gastric contents revealed no free acid. Sternal bone marrow studies were normal. A dermatologic consultant[5] diagnosed the above-described skin lesion as acanthosis nigricans, and stated emphatically that it was always associated with adenocarcinoma. On January 20, 1953, bronchoscopy revealed nothing unusual. On January 23, 1953, a *right* scalene node biopsy yielded tissue containing undifferentiated carcinoma with signet cells. The consensus of opinion was that the primary malignancy lay in the pancreas. The patient died in March, 1953, and autopsy revealed carcinoma of the head of the pancreas.

Carcinoma of the cervix—Case 5. The patient, S. R., was a 42-year-old Latin-American female. While being treated by radiation therapy for carcinoma of the cervix, stage III, this patient developed pain in the right upper quadrant and chest. A palpable liver was noted and she developed bilateral hilar enlargement with pulmonary infiltrations, as shown by roentgenographic study (figure 22). Examination of a *right* scalene node on September 24, 1952, revealed squamous cell carcinoma.

Indications for Scalene Node Biopsy

In general, the indications for scalene node biopsy may be stated in an exaggeratedly simple manner by advising that this biopsy be done whenever it is advisable to know the contents of the mediastinal nodes. In detail, the indications are as follows: Scalene node biopsy is of value in all cases of proved bronchogenic carcinoma in order to uncover at least a portion of the cases in which the carcinoma has extended beyond the immediate confines of the chest. It should be done in all cases of suspected bronchogenic carcinoma. This will actually include all roentgenographic opacities not otherwise identified, especially the small, isolated, often peripheral, asymptomatic lesions. In instances of hilar and/or mediastinal roentgenographic enlargement, scalene node dissection will reveal many cases of Boeck's sarcoid, enlarged tuberculous mediastinal nodes, lymphosarcoma, and Hodgkin's disease. Cultures of scalene nodes can result in isolation of specific fungi in many cases of pulmonary fungal infection not otherwise diagnosed. The value of isolation of tubercle bacilli from these nodes in the diagnosis of a tuberculous pulmonary parenchymal lesion remains to be proved. In cases of pulmonary infiltrates or infiltrations suspected of being metastatic tumors, scalene node biopsy material may reveal the exact microscopic type of tumor and, in conjunction with clinical findings, may lead to the prompt detection of the primary site of the neoplasm. In such cases, in the absence of autopsies, if the procedure had no other value, it should improve the accuracy of vital statistics. Finally, scalene node dissection should be of value in cases of medical-legal interest (pneumoconiosis) wherein the offending foreign-body substances may be identified.

SUMMARY

The value of and indications for scalene node biopsy have been presented, based on a series of 187 patients upon whom the procedure was performed. In 67

[5] Dr. James H. Strauch, civilian consulting dermatologist.

of the patients, examination of the biopsy material yielded convincing evidence of the identity of associated intrathoracic disease which had previously been undiagnosed despite application of the generally used nonsurgical diagnostic measures. The disease processes found in the scalene nodes in the present series included Boeck's sarcoid, bronchogenic carcinoma, tuberculosis, lymphosarcoma, Hodgkin's disease, fungal infections, and carcinoma which had metastasized to the lung.

SUMARIO

Biopsia de los Ganglios Escalenos

Expónense aquí el valor y las indicaciones de la biopsia de los ganglios escalenos, a base de una serie de 187 enfermos en quienes se ejecutó tal procedimiento. En 67 de los enfermos, el examen del material de la biopsia aportó datos convincentes en cuanto a la identidad de la enfermedad intratorácica presente, la cual no había sido diagnosticada antes, a pesar del empleo de las providencias no quirúrgicas usadas generalmente para el diagnóstico. Los procesos morbosos descubiertos en los ganglios escalenos en la serie actual comprendieron: sarcoide de Boeck, carcinoma broncógeno, tuberculosis, linfosarcoma, enfermedad de Hodgkin, micosis y carcinoma que había metastatizado al pulmón.

RESUME

Biopsie d'un ganglion sus-clavicularire ("scalène node")

La valeur et les indications de la biopsie d'un ganglion sus-claviculaire "scalène node" sont présentées. Ces notions sont fondées sur 187 observations de malades chez lesquels ce procédé a été appliqué. Chez 67 de ces sujets, l'examen du fragment a fourni des indications certaines sur l'identité de l'affection intrathoracique associée, alors que celle-ci n'avait pu être diagnostiquée en dépit de la mise en oeuvre des méthodes diagnostiques non chirurgicales usuelles. Dans les études ci-dessus, les processus pathogènes découverts dans les ganglions situés au niveau du scalène comprenaient le sarcoïde de Boeck, le cancer bronchiogénique, la tuberculose, le lymphosarcome, la maladie de Hodgkin, les mycoses et un cancer métastatique du poumon.

REFERENCES

(1) Daniels, A. C.: A method of biopsy useful in diagnosing certain intrathoracic diseases, Dis. of Chest, 1949, *16*, 360.

(2) Storey, C. F., and Reynolds, B. M.: Biopsy techniques in the diagnosis of intrathoracic lesions, Dis. of Chest, 1953, *23*, 357.

(3) Weiss, H. A., Campbell, R. M., and Budenz, G. C.: Sarcoidosis diagnosed by means of subclavian lymph node biopsy: Report of a case, J.A.M.A., 1951, *146*, 1221.

(4) Johnson, J. E., Jr., and MacCurdy, J. M.: Pulmonary histoplasmosis diagnosed by scalene node biopsy, Am. Rev. Tuberc., 1952, *66*, 497.

(5) Seabury, J.: Personal communication.

(6) Kligman, A. M., Mescon, H., and DeLamater, E. D.: Hotchkiss-McManus stain for the histopathologic diagnosis of fungus diseases, Am. J. Clin. Path., 1951, *21*, 86.

A STUDY OF THE EFFECTS OF ISONIAZID ON THE EMOTIONS OF TUBERCULOUS PATIENTS[1]

THOMAS H. LORENZ, GEORGE CALDEN, AND JOSEPH L. OUSLEY

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INTRODUCTION

In the first reports on the use of the hydrazine derivatives of isonicotinic acid in the treatment of human tuberculosis, note was made of striking change in the patient's "sense of well-being" (1, 2, 3). This change was manifested primarily by elevation of mood (euphoria) and an increase in psychomotor activity, even to the point of precipitating an unusual increase in the number of disciplinary problems among these patients (2). Emergence of such altered affect and behavior has been noted to occur, in many instances, within a day or two after starting therapy. In one series, remarkable improvement in sense of well-being was seen in *all* patients by the second or third week of treatment (3).

Subsequently, other investigators have observed "euphoria" in tuberculosis patients who received isoniazid (4), which tends to confirm the previous clinical accounts. On the other hand, there also have been contrary reports (5). Similarly, in the treatment of psychotic tuberculous patients with these agents, one group of workers reported that marked improvement occurred in the mental status of their subjects (5), whereas another group found no significant change (5).

Obviously, the above-noted conflicting reports, derived from clinical observations, still leave unanswered the question whether or not euphoria, or some other emotional change, actually does occur with use of the hydrazine derivatives of isonicotinic acid. The purpose of this study, therefore, was to determine what effect, if any, isoniazid therapy had on the emotions of tuberculous patients.

Since it is notoriously difficult to assess problems of this type on clinical grounds alone, in the present investigation methods which permitted objective evaluation and measurement of emotional status were used, in addition to clinical appraisal of patients.[2]

MATERIALS AND METHODS

Subjects

Fifteen male patients, who were selected at random for inclusion in the isoniazid therapy program, were the subjects in this study. These patients had all recently been admitted to the Veterans Administration Hospital, Madison, Wisconsin, and had been shown to have active pulmonary tuberculosis. The age range of the group was from twenty-two to sixty-seven years (mean age, forty-

[1] From the Psychosomatic Section of the Medical and Tuberculosis Service, Veterans Administration Hospital, Madison, Wisconsin.

[2] Since iproniazid was not used in the present study, the observations and conclusions derived from this investigation do not necessarily apply to this derivative of isonicotinic acid.

two and one-half years). One of the subjects was a Negro, the remainder were white. Eight of the patients had far advanced disease, 5 were diagnosed as having moderately advanced disease, and 2 had minimal tuberculosis. All of the patients received isoniazid in a dosage of 100 mg. three times daily. In addition to this, 8 of the subjects were given streptomycin, 1 gm. twice weekly, and 5 others received para-aminosalicylic acid (PAS), 12 gm. daily. The group was composed of patients who were residents (in approximately equal numbers) of four different wards of the hospital.

Procedure

Promptly after presentation to the therapy board, and before isoniazid therapy was begun, each patient was seen by the investigators in order to obtain the following data:

> An introspective statement by the patient estimating his own mood and state of well-being; a clinical evaluation of the patient's current emotional status by a trained medical observer; an evaluation of the patient's mood and attitude by psychological testing; and a pain threshold determination.

Immediately thereafter, isoniazid therapy was instituted. After two weeks (7 patients) or three weeks (8 patients), the subject's emotional status was again evaluated, both introspectively and objectively. Psychological tests and pain threshold measurements were repeated as before.

When both sets of observations had been completed for each of these patients, the data obtained before therapy was compared with those obtained during isoniazid treatment. Statistical analysis for significance of differences was carried out on the results derived from the psychological tests and pain threshold determinations during these two periods.

Methods

Psychological tests: The Guilford Inventory of Factors STDCR and the Madison Sentence Completion Form were the two psychological techniques used to measure the patient's mood changes.

The Guilford Inventory (6) has been shown to be an adequate indicator of the extent to which a person is depressed or cheerful, introverted or extroverted, happy-go-lucky or serious-minded, and flighty or stable. The test consists of 175 questions of the "yes-?-no" type which permit scoring on five traits of mood and emotionality. These traits are described in the manual as follows:

S—*Social introversion-extroversion.*—Shyness, seclusiveness, tendency to withdraw from social contacts, versus sociability, tendency to seek social contacts and to enjoy the company of others.

T—*Thinking introversion-extroversion.*—An inclination to meditative or reflective thinking, philosophizing, analysis of one's self and others, versus an extrovertive orientation of thinking.

D—*Depression.*—Habitually gloomy, pessimistic mood, with feelings of guilt and unworthiness, versus cheerfulness and optimism.

C—*Cycloid disposition.*—Strong emotional fluctuations, tendencies toward flightiness and emotional instability, versus uniformity and stability of moods, evenness of disposition.

R—*Rhathymia.*—A happy-go-lucky, carefree disposition, liveliness, impulsiveness, versus an inhibited, over-controlled, conscientious, serious-minded disposition.

If isoniazid exerts a euphoric effect upon patients, it should be reflected in the Guilford Inventory test results by sharp decreases in social-introversion (S), thinking-introversion (T), and depression (D) scores, and elevations in cycloid disposition (C) and rhathymia (R) scores.

The Madison Sentence Completion Form (7) is designed to evaluate the attitudes and emotional reactions of tuberculosis patients. The form consists of eighty unfinished sentences presented to the patient for completion, for example, "Lying in bed makes me . . .," "When a person is first told he has tuberculosis . . .," et cetera. It has been found that by completing these sentences, the patient expresses his own wishes, desires, fears, hopes, and expectations in regard to his disease and his hospitalization. Five of the eighty items were selected for analysis in this study, since these particular items yield responses indicative of the patient's feeling of well- or ill-being, contentment or discontentment, and bodily comfort or discomfort. The five items are:

1. The future looks . . .
2. The chances of my getting better . . .
3. Ten years from now . . .
4. I feel I am getting . . .
5. The longer I remain in bed . . .

The patient's sentence completion responses to each of the items were rated on a 5-point dysphoria-euphoria scale. Responses such as "the future looks . . . *bright and wonderful*" and "I feel I am getting . . . *the best medical care in the whole U.S.A.*" were rated 5 since they are indicative of a high degree of mood elevation. On the other hand, a rating of 1 for dysphoric mood was assigned to such responses as "the future looks . . . *gloomy and miserable*" or "the longer I remain in bed . . . *the lousier I feel.*" Completed sentences that reflected moderate degrees of dysphoria or euphoria were rated 2, 3 and 4, respectively. Comparisons of ratings for the two sets of sentence completion responses (before and during isoniazid treatment) were used to determine the extent of the patient's change in mood.

Pain threshold determinations: The Hardy-Wolff-Goodell pain threshold apparatus (8) is a device which delivers a controlled, measured amount of radiant therapy to a specially prepared skin surface so that the level of pain perception and pain reaction can be determined. By using a pair of lenses, the light from a rheostatically controlled 500-watt incandescent lamp is focused through a fixed aperture onto the skin-test site. An electrically operated shutter provides for a standard exposure time of three seconds. An absorption coefficient of unity is ensured by blackening the skin with india ink sufficiently long before testing to allow for complete drying.

A voltmeter is attached across the lamp circuit. The voltage readings are recorded when the subject first perceives pain and again when he reacts to it. Since the energy output for any given voltage is known, the voltmeter readings are later translated into energy units: gram calories per second per square centimeter.

In the present investigation, all subjects were given similar instructions prior to testing. They were informed what the testing device was and how it operated. They were told that sometime during the series of trials they would feel a sharp, stinging, pricking, or jabbing sensation (sometimes described as a "hot needle") and that, as soon as they perceived this, they were to comment on it. It was pointed out that the sensation of warmth was *not* the desired end point. Reaction to the stimulus by wincing or withdrawing was not mentioned before the experiment. Ordinarily a complete experiment on one person entailed ten to fifteen exposures of varying intensity of stimulation using the mid-forehead and dorsum of the hand alternately. All of the tests were performed by the same operator and, as nearly as possible, under basal conditions. None of the patients received analgesics on the day of testing.

The pain *perception* threshold was taken as the lowest level of stimulation at which the

subject experienced a sharp, stinging, or pricking sensation; whereas the pain *reaction* threshold was considered as the lowest level at which a motor response was noted, i.e., a wince or a withdrawal.

Pain threshold has been shown to be altered by emotional reactions (9, 10, 11), inattention, and distractability (12) of the subject. It also is influenced by appropriate suggestion

TABLE 1

SUMMARY OF CLINICAL DATA

PATIENT	AGE	DIAGNOSIS	DRUG THERAPY ISONIAZID 100 MG. THREE TIMES DAILY PLUS:	DURATION	WEIGHT CHANGE	MOOD CHANGE	
						Patient's Estimate	Doctor's Impression
	years			*days*	*kilograms*		
WJ	67	Far advanced	PAS, 12 gm./day	21	+5	Much improved	Improved
LL	50	Far advanced	PAS, 12 gm./day	21	±	Improved	Same
SC	28	Minimal	Streptomycin, 1 gm. twice weekly	21	±	Same	Same
KW	31	Moderately advanced	—	21	+5	Improved	Same
KR	29	Moderately advanced	Streptomycin, 1 gm. twice weekly	21	+3	Improved	Same
EA	62	Far advanced	—	21	+6	Improved	Same
LJ	22	Moderately vanced	PAS, 12 gm./day	21	±	Same	Same
MJ	30	Minimal	Streptomycin, 1 gm. twice weekly	21	±	Same	Same
BW	63	Far advanced	PAS, 12 gm./day	14	+3	Improved	Same
BR	42	Moderately advanced	PAS, 12 gm./day	14	+3	Improved	Same
KJ	29	Moderately advanced	Streptomycin, 1 gm. twice weekly	14	±	Improved	Same
HR	60	Far advanced	Streptomycin, 1 gm. twice weekly	14	−5	Worse	Worse
JE	44	Far advanced	Streptomycin, 1 gm. twice weekly	14	−1	Same	Same
PW	58	Far advanced	Streptomycin, 1 gm. twice weekly	14	±	Same	Worse
OB	23	Far advanced	Streptomycin, 1 gm. twice weekly	14	±	Worse	Worse

and placebos (12). Thus, it seemed likely that if euphoria were produced by isoniazid, either directly or indirectly, a significant difference in the pain threshold values during treatment would be demonstrated in the patients in this series.

RESULTS

Clinical Observations

General: As may be seen in table 1, 6 of the patients gained 3 to 6 kilograms in weight during the fourteen- to twenty-one-day interval between the time of

starting isoniazid therapy and re-evaluation. Generally, these patients also experienced increase in appetite and reduction in cough and/or expectoration. Seven patients showed no significant change in weight or in other signs, while 2 of the subjects lost 1 and 5 kilograms, respectively, during the period of observation. One of the 2 patients who lost weight showed an increase in ulcer symptoms with moderate epigastric discomfort, anorexia, nausea, and gaseousness shortly after starting isoniazid treatment. The other patient offered no new physical complaints during the period of study.

Patient's estimate of mood and well-being: In general, there was a positive relationship between improved weight, appetite, and other clinical signs on the one hand, and improvement in the patient's mood and "sense of well-being" on the other. Two of the patients stated that they felt much better during treatment than they had before. They indicated that they were definitely more hopeful and cheerful, less restless and dejected while on isoniazid than prior to use of this agent. Six of the patients felt a little improved in mood and well-being. Five of the patients were of the opinion that they felt the same during treatment, as compared with before, whereas two patients felt worse when receiving isoniazid. One of the latter patients who felt worse was the person who had the exacerbation of ulcer symptoms during therapy. Accompanying his somatic complaints were psychic changes consisting of irritation, grouchiness, increasing anxiety, and discouragement. The other patient who felt worse offered no specific complaints but just felt "kind of down in the mouth" and "moody for no reason at all" while on the drug.

Examiner's evaluation of emotional status: Comparison of the patient's emotional status before and during isoniazid therapy, based on the clinical evaluation of a trained medical observer, revealed that one patient's mood was distinctly elevated, 11 showed no change, and 3 had a more unfavorable affect in one way or another (table 1).

The one patient who improved emotionally was *not* considered to be euphoric but still was definitely more cheerful, talkative, outgoing, and confident than before treatment. It was this patient who had experienced a gain of 5 kilograms in weight, improved appetite, and regarded himself as much improved in mood after therapy had begun. The seven other persons who regarded themselves as being improved in mood were essentially unchanged in this regard in the examiner's opinion.

Four of the 5 patients who felt the same also appeared unchanged to the examiner. The fifth subject, who reported feeling the same showed some evidence of apathy and depression which had not been apparent in the initial evaluation. The two persons who regarded themselves as being worse (emotionally and/or physically) while on treatment were also regarded as worse by the examiner.

Psychological Tests

Guilford Inventory: The Guilford Inventory test-retest results, as indicated in table 2, did not reveal striking changes of mood attendant upon the administration of isoniazid. The differences in mean scores for each of the five factors showed only slight alteration of affect. It is interesting, however, that the

changes that did appear for three of the five factors were in the anticipated direction. The small decreases in social-introversion (S), thinking introversion (T), and depression (D) scores indicated that the patients may have become slightly less seclusive, less meditative, and less depressed, and generally more socially outgoing and cheerful. The unanticipated minor decline in mean cycloid disposition (C) scores suggested that the patients might have become a little less

TABLE 2

COMPARISON OF GUILFORD INVENTORY STDCR SCORES OBTAINED BEFORE AND DURING ISONIAZID TREATMENT

PATIENT	FACTORS									
	S		T		D		C		R	
	Before	During	Before	During	Before	During	Before	During	Before	During
WJ	13	11	19	12	6	5	10	7	46	45
LL	17	17	18	17	6	4	9	3	34	32
SC	15	15	15	11	9	6	9	6	30	29
KW	7	8	36	32	17	11	27	17	45	47
KR	11	13	29	30	11	12	17	19	36	33
EA	12	8	19	19	5	7	4	9	26	39
LJ	8	6	22	21	10	8	20	17	47	50
MJ	17	14	24	25	17	11	28	11	48	43
BW	22	14	20	22	5	5	6	4	38	40
BR	33	30	23	34	38	43	51	54	33	31
KJ	8	8	43	29	21	11	39	26	54	55
HR	11	13	27	20	11	16	12	12	50	44
JE	30	32	31	37	35	41	48	47	54	41
PW	18	16	38	37	12	12	22	18	47	48
OB	16	10	41	45	16	11	12	14	33	52
Mean	15.87	14.33	27.00	25.40	14.60	13.53	20.93	17.60	41.40	41.93
Sigma	7.36	7.26	8.66	8.45	9.66	11.62	14.50	14.32	8.71	7.71
t*	1.77		1.00		0.89		1.29		0.27	

* None of the t values are significant at the 0.05 level.

flighty and more stable in disposition. However, these minor changes were not conclusive, since none of these differences in mean test-retest scores assumed statistical significance (at the 0.05 level of confidence). It is quite apparent, therefore, that mood changes of a magnitude sufficiently striking to be called euphoric were not reflected in the Guilford Inventory responses.

Analysis of the test-retest results on the five items of the Madison Sentence Completion Form did not reveal any consistent alteration in mood or attitude. As indicated in table 3, the differences in group mean ratings of responses for each sentence (before and during isoniazid treatment) were quite small. None of these differences in test-retest scores achieved statistical significance at the

0.05 confidence level. A further analysis of changes in test-retest sentence completion ratings for each individual patient also yielded negative results. The responses of 7 of the 15 patients showed an absence of any measurable change in mood, either in the direction of euphoria or dysphoria. Four of the patients revealed a small increase in well-being, whereas 4 others indicated a comparable decrease in euphoric mood. Again, in none of the subjects were there striking changes in affect or attitude.

TABLE 3

COMPARISON OF RATINGS OF SENTENCE COMPLETION RESPONSES OBTAINED BEFORE AND DURING ISONIAZID TREATMENT

PATIENT	SENTENCES									
	1		2		3		4		5	
	Before	During	Before	During	Before	During	Before	During	Before	During
WJ	4	4	3	4	4	3	2	2	4	4
LL	2	4	4	3	3	3	3	3	4	4
SC	4	2	4	4	4	4	2	3	4	4
KW	2	2	4	5	4	4	4	4	4	4
KR	2	4	4	4	4	4	4	4	2	4
EA	5	3	5	5	5	5	5	3	5	5
LJ	2	4	3	4	5	5	4	4	4	4
MJ	2	4	4	3	5	4	3	3	4	4
BW	3	3	3	4	4	4	3	2	5	4
BR	4	3	4	2	3	4	3	2	3	4
KJ	4	4	4	5	4	5	5	5	4	4
HR	2	2	2	1	2	2	1	1	3	1
JE	4	5	4	4	4	4	3	3	4	4
PW	2	2	4	3	5	5	2	2	4	3
OB	4	2	1	1	5	4	3	3	4	4
Mean	3.07	3.20	3.53	3.47	4.00	3.13	3.13	2.93	3.80	3.80
Sigma.....	1.06	0.98	0.96	1.26	0.85	0.82	1.09	1.00	0.72	0.84
t*.....	0.35		0.15		0.23		0.53		0.00	

* None of the t values are significant at the 0.05 level.

Pain Threshold Determinations

As shown in table 4, the mean pain *perception* threshold of the group before isoniazid therapy was 0.302 gm.cals. per sec. per cm². During treatment it was 0.306 gm.cals. per sec. per cm².

The pretreatment mean pain *reaction* threshold of these patients was 0.464 gm.cals. per sec. per cm²., whereas during therapy its value was 0.477 gm.cals. per sec. per cm².

Statistical analysis yielded no significant differences in the means before and during isoniazid treatment.

TABLE 4

COMPARISON OF PAIN THRESHOLD DETERMINATIONS OBTAINED BEFORE AND DURING ISONIAZID TREATMENT

PATIENT	PERCEPTION THRESHOLD*		REACTION THRESHOLD*	
	Before Isoniazid	During Isoniazid	Before Isoniazid	During Isoniazid
WJ	0.288	0.252	0.480	0.365
IL	0.326	0.326	0.555	0.555
SC	0.228	0.326	0.555	0.555
KW	0.252	0.288	0.404	0.365
KR	0.326	0.326	0.404	0.480
EA	0.252	0.252	0.326	0.442
LJ	0.252	0.288	0.326	0.326
MJ	0.326	0.326	0.480	0.404
BW	0.326	0.365	0.555	0.555
BR	0.365	0.365	0.555	0.555
KJ	0.288	0.252	0.442	0.480
HR	0.288	0.365	0.365	0.520
JE	0.365	0.252	0.480	0.480
PW	0.326	0.326	0.480	0.520
OB	0.326	0.288	0.555	0.555
Mean	0.302	0.306	0.464	0.477
Sigma	0.045	0.045	0.094	0.078
t†	0.24		0.43	

* In gm. cals./sec./cm.²

† None of the t values are significant at the 0.05 level.

DISCUSSION

In the present experimental study, four separate indices of the patient's emotional status were utilized to investigate mood changes associated with isoniazid treatment. Neither consistent nor significant emotional changes were found in this series of 15 tuberculous patients who were selected at random and treated with isoniazid in conservative dosage. None of these subjects became euphoric.

These negative findings are not unique. The absence of euphoria during isoniazid treatment has been reported by others in the past (5). Also, at the Madison VA Hospital it has been observed clinically in more than 150 patients treated with isoniazid that mood changes of sufficient magnitude to be labeled euphoric have not occurred during the course of therapy (13). These observations, however, have been confined to clinical evaluation and have been uncontrolled. The present study, although limited to 15 subjects, provides controlled experimental observations which support these negative clinical impressions gained from experience with larger series of patients.

It is known that isoniazid and its derivatives may induce toxicity, both in the experimental animal (14) and in man (3, 15), manifested by stimulation

and/or hyper-irritability of the nervous system. Such a toxic effect on the central nervous system conceivably might be responsible for the pathologic *elevation* of mood (euphoria) seen in patients treated with these drugs. The absence of significant emotional change noted in this study, as well as in several former ones (5), however, suggests that this possibility is quite unlikely.

Mood elevation with newly introduced therapeutic agents is not unusual. When streptomycin, for example, was first used in the treatment of tuberculosis, elation of patients was quite commonly seen (16). Most physicians recognize that mood changes associated with new forms of treatment often can be ascribed to the potent, though subtle, influences of suggestion and placebo effect. Probably isoniazid and its derivatives have been no exception to this general rule.

If the euphorigenic properties attributed to isoniazid are largely a reflection of such indirect psychological factors, then the question arises why they were not operative in producing significant emotional changes in the present series. The explanation for this might be found in some of the notable differences between this study and those in the past in which euphoria was observed. First, and in contrast to the random selection of patients studied in the present investigation, the sample of persons treated with isoniazid and its derivatives by earlier workers was a *selected* one, largely made up of patients who were exceedingly ill (1, 2). It is now well documented that these drugs are effective antimicrobial agents, and particularly so with acutely and seriously diseased tuberculous patients. It would not be surprising, therefore, that a group of pre-moribund persons would become relatively elated and hopeful secondary to the physical improvement they experienced when treated with these agents. In a less selected series of patients, on the other hand, this would not be so likely to occur.

Furthermore, it is pertinent to note that this investigation was undertaken approximately one year after isoniazid and its derivatives were first tried in human tuberculosis. With the passage of time there has been a lessening of the enthusiasm and fanfare associated with their introduction. In contrast with the emotionally charged atmosphere under which these agents previously were prescribed, isoniazid is administered at this hospital in a rather matter-of-fact and routine fashion under the randomization treatment program of the Veterans Administration. The vast majority of patients here are not under the impression that they are being given a miracle drug. At this hospital most of the patients have a fairly realistic attitude toward drug therapy, and treatment with these agents is attended by a minimum of environmental suggestion.

There is an additional consideration in regard to possible explanations of the euphoria found in the past but not in the present study. It is noteworthy that, on *introspective* appraisal, more than one half of the subjects in this series believed that they improved somewhat after receiving isoniazid. In contrast to this, a trained observer found distinct elevation in affect in only one patient, and the results obtained by objective means (psychological tests and pain threshold measurements) showed no significant change in the emotional reactions of the group. Thus, the more objective the method of evaluation, the less evident became any significant alteration in the patient's mood. By this token, it seems

possible that the emotional changes noted in several earlier clinical reports and based primarily on subjective estimates may have been more apparent than real.

There is reason to believe that the personality characteristics, attitudes toward their disease, and adjustment to hospitalization of individual patients have relevance to the emotional reactions that may occur in some patients coincidental with isoniazid treatment. In this series, several patients experienced minor improvement in mood. Others remained essentially unchanged from an emotional standpoint, and a few of the subjects showed a less favorable affect when on isoniazid therapy. Even though these deviations were largely subjective and only minor in degree, their occurrence suggests that a patient's emotional reactions when receiving isoniazid are more a reflection of the individual patient than of the drug per se.

SUMMARY

As a consequence of conflicting reports in the literature, the present study was undertaken to investigate the effect of isoniazid therapy on the emotions of tuberculous patients. Fifteen subjects with active pulmonary disease were selected at random for this purpose. The mood and emotional status of the patients were evaluated before and during drug treatment by using four different techniques: (*a*) the patient's introspective estimate, (*b*) a trained examiner's impression, (*c*) psychological tests, i.e., the Guilford Inventory of Factors STDCR and the Madison Sentence Completion Form, and (*d*) a pain-threshold determination.

Comparison of these data, obtained before and during isoniazid treatment, revealed that no significant changes occurred in the mood of the patients in this series. None of the patients became euphoric.

The negative findings in this study are compared with the results of earlier reports in which euphoria was observed. The differences in findings are discussed in terms of certain factors which may have contributed to the positive results of earlier investigators.

It is proposed that suggestion or placebo effect, as a consequence of situational influences within the treatment setting and/or the individual patient's attitudes, rather than the drug per se, might have been responsible for the mood changes previously noted in patients treated with isoniazid and its derivatives.

SUMARIO

Estudio de los Efectos de la Isoniacida sobre las Emociones de los Tuberculosos

A consecuencia de los informes contradictorios que constan en la literatura, se emprendió el estudio actual para investigar el efecto de la isoniacidoterapia sobre las emociones de los tuberculosos, escogiéndose para ello a 15 sujetos con afección pulmonar activa. El estado de ánimo y afectivo de los enfermos fué justipreciado antes de la farmacoterapia y durante ésta por medio de cuatro técnicas distintas: (*a*) estimación introspectiva del enfermo; (*b*) impresión formada por un examinador adiestrado; (*c*) pruebas psicológicas, es decir, el Inventario Guildord de los Factores STDCR y el Formulario Madison para Completar Oraciones y (*d*) una determinación del umbral para el dolor.

La comparación de esos datos, obtenidos antes de la isoniacidoterapia y durante ésta,

reveló que no había habido modificaciones importantes en el estado de los enfermos de esta serie. Ninguno de ellos se volvió eufórico.

Compáranse los hallazgos negativos de este estudio con los presentados en trabajos anteriores, en que se observó euforia. Discútense las diferencias en los resultados en términos de ciertos factores que pueden haber intervenido en los resultados positivos de los investigadores anteriores.

Apúntase que el efecto de la sugestión o del placebo, a consecuencia de factores circunstantes dentro del cuadro del tratamiento y/o la actitud del enfermo dado, más bien que la droga por sí misma, tal vez fueran la causa de los cambios del estado de ánimo observados anteriormente en los sujetos tratados con isoniacida y sus derivados.

RESUME

Une étude des effets de l'isoniazide sur les reactions émotionnelles des tuberculeux

A la suite de la publication de rapports contradictoires, l'étude actuelle a été entreprise pour déterminer l'effet de l'isoniazide sur le psychisme du tuberculeux soumis à ce traitement. Dans ce but, quinze sujects atteints de tuberculose en évolution ont été choisis au hasard. Leur comportement, leur réactions émotionnelles ont été determinées avant et au cours du traitement en utilisant quatre méthodes différentes: (*a*) l'observation introspective du malade, (*b*) l'impression du psychologue qualifié, (*c*) les tests psychologiques, a.d., le "Guilford Inventory of Factors STDCR" et la "Madison Sentence Completion Form," (*d*) la détermination du seuil de la douleur.

La comparaison de ces données, obtenues avant et pendant l'administration de l'isoniazide, a revélé qu'aucune modification de l'état psychique du malade ne s'était produite chez les sujects de ce groupe. L'euphorie ne s'est manifestée chez aucun de ces malades.

Les résultats négatifs obtenus dans cette étude ont été comparés avec ceux des rapports antérieurs, dans lesquels l'euphorie avait été observée. Les différences entre ces résultats sont étudiées en tenant compte de certain facteurs qui pourraient avoid influencé les résultats positifs des investigateurs précédents.

L'auteur suppose que la suggestion ou l'effet du "placebo," comme conséquence des influences particulières exercées par les conditions du traitement et par le comportement individuel du malade, ou par l'un ou l'autre de ces facteurs, plutôt que l'action du medicament en soi, peut avoir été responsable des modifications du psychisme observées précédemment chez les malades traités par l'isoniazide et ses dérivés.

REFERENCES

(1) Robitzek, E. H., Selikoff, I. J., and Ornstein, G. G.: Chemotherapy of human tuberculosis with hydrazine derivatives of isonicotinic acid, Quart. Bull., Sea View Hosp., 1952, *13*, 27.

(2) Robitzek, E. H., and Selikoff, I. J.: Hydrazine derivatives of isonicotinic acid (Rimifon, Marsilid) in the treatment of active progressive caseous—pneumonic tuberculosis, Am. Rev. Tuberc., 1952, *65*, 402.

(3) Selikoff, I. J., and Robitzek, E. H.: Tuberculosis chemotherapy with hydrazine derivatives of isonicotinic acid, Dis. of Chest., 1952, *21*, 21.

(4) Quarterly Progress Report of the VA-Army-Navy study on the chemotherapy of tuberculosis, July, 1952.

(5) Quarterly progress report of the VA-Army-Navy study on the chemotherapy of tuberculosis, October, 1952.

(6) Guilford, J. P.: Inventory of factors STDCR, Beverly Hills, California, Sheridan Supply Co., 1940.

(7) Calden, G.: A method for evaluating the attitudes of tuberculous patients, Am. Rev. Tuberc., 1953, *67*, 6.

(8) Hardy, J. D., Wolff, H. G., and Goodell, H.: Studies on pain: New method for measuring pain threshold; observations on spatial summation of pain, J. Clin. Investigation, 1940, *19*, 649.

(9) Chapman, W. P.: Measurements of pain sensitivity in normal control subjects and in psychoneurotic patients, Psychosom. Med., 1944, *6*, 252.
(10) Schilling, R. F., and Musser, M. J.: Pain reaction thresholds in psychoneurotic patients, Am. J. M. Sc., 1948, *215*, 195.
(11) Lorenz, T. H., Olson, D. E., and Musser, M. J.: A study of pain threshold in tuberculous patients, Am. Rev. Tubere., 1952, *66*, 449.
(12) Wolff, H. G., and Goodell, H.: The relation of attitude and suggestion to the perception of and reaction to pain, A. Research Nerv. & Ment. Dis., Proc. (1942), 1943, *23*, 434.
(13) Unpublished data.
(14) Rubin, B., Hassert, G. L., Jr., Thomas, G. H. B., and Burke, J. C.: Pharmacology of isonicotinic acid hydrazide (Nydrazid), Am. Rev. Tubere., 1952, *65*, 392.
(15) Selikoff, I. J., Robitzek, E. H., and Ornstein, G. G.: Toxicity of hydrazine derivatives of isonicotinic acid in the chemotherapy of human tuberculosis, Quart. Bull., Sea View Hosp., 1952, *13*, 17.
(16) Thomas, M. C.: Personal communication.

ISONIAZID AND ISONIAZID—PARA-AMINOSALICYLIC ACID IN THE TREATMENT OF TUBERCULOUS SINUSES AND FISTULAS[1]

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INTRODUCTION

The favorable effect of streptomycin in the healing of sinuses and fistulas of tuberculous origin has been reported by several writers (1, 2, 3). When the antituberculous action of isoniazid was reported early in 1952 (4), it was important to ascertain whether this drug might also be of value in the treatment of tuberculous sinuses and fistulas. The emergence of drug-resistant tubercle bacilli has proved to be a serious handicap to streptomycin therapy in all types of tuberculous lesions. Isoniazid had been found to be effective in the treatment of patients with pulmonary tuberculosis whose bacilli were resistant to streptomycin. It would be of great advantage if a similar beneficial effect could be demonstrated in patients with sinuses and fistulas caused by tubercle bacilli which were resistant to streptomycin.

OBSERVATIONS

Twenty-seven patients with tuberculous sinuses and fistulas were treated with isoniazid from February to December, 1952. Fifteen patients had bronchopleurocutaneous fistulas; 11 had pleurocutaneous sinuses; and one had sinuses associated with tuberculous lymph nodes. The patients ranged in age from twenty-four to fifty years. Twenty were white and 7 were nonwhite. There was only one female patient in the series. All of the chest wall sinuses or fistulas had followed some type of thoracic surgery and had been present from one month to ten years before isoniazid treatment was started. Seven of the bronchopleurocutaneous fistulas and 5 of the pleurocutaneous sinuses had appeared following pneumonectomy (table 1). Five of the fistulas and 4 of the sinuses followed total or segmental lobectomy. Cavernostomy, spontaneous pneumothorax, and thoracoplasty had preceded the appearance of the fistulas or sinuses in the other cases. All of the patients had previously received streptomycin and para-aminosalicylic acid (PAS) for at least one hundred and twenty days. Three had received pyrazinamide or viomycin in addition to streptomycin. Nearly all of the patients received penicillin, terramycin, or aureomycin at some time in their course before isoniazid therapy was initiated.

Treatment Regimen

One patient was given 300 mg. of isoniazid daily; the others received 150 mg. per day. One patient died after eighteen days of treatment. Death was not attributed to the effects of the drug. The remaining patients were treated for periods ranging from two to ten months. During the latter part of the study, 12

[1] From the Veterans Administration Hospital, Oteen, North Carolina.

gm. of PAS were given daily, in addition. This was done after reports of the isolation of isoniazid-resistant tubercle bacilli appeared in the literature. The average duration of treatment for fistulas was six months; and for sinuses, four months. The wounds were dressed daily. Irrigations or packs with streptokinase and streptodornase were used to remove debris in a few patients. The sinuses and fistulas were delineated by lipiodol injections and roentgenographic examination. If circuitous tracts or "dead space" were present, auxiliary surgical procedures such as thoracoplasty, an unroofing operation, or further resection were done. Such measures were necessary in 9 of the patients. One man with a bronchoesophagocutaneous fistula had a temporary jejunostomy done for feeding. He made a remarkable recovery and obtained a complete closure of the fistula and chest wound.

TABLE 1

ANATOMIC CAUSE OF TUBERCULOUS FISTULAS AND SINUSES

	BRONCHOCUTANEOUS FISTULAS	PLEUROCUTANEOUS SINUSES
Pneumonectomy	7	5
Lobectomy	2	4
Segmental lobectomy	3	0
Cavernostomy	2	0
Spontaneous pneumothorax	1	1
Thoracoplasty	0	1
Total	15	11

Evidence of drug toxicity was seen in only one patient, who developed jaundice after one month of therapy. This was attributed to a post-transfusion hepatitis. After the jaundice had cleared, isoniazid was restarted and continued for three months without untoward incident. He then developed a moderate granulocytopenia (24 per cent neutrophils). After discontinuing isoniazid, the neutrophil count became normal.

Results of Treatment

Following treatment, the 27 patients were classified as either healed or unhealed (table 2). The unhealed group was further classified as improved or unimproved. Eighteen patients attained healing, and in 9 the lesions did not heal. Seven of the patients with unhealed sinuses, however, experienced considerable improvement of their lesions; 9 of the patients with bronchocutaneous fistulas attained healing; and in 6 patients the lesions failed to heal. Five of the latter lesions were improved, however, and one patient with an unhealed fistula died. In 9 of the patients with pleurocutaneous sinuses, the process healed, and in 2 it remained unhealed. The patients who did not experience complete healing usually showed considerable improvement in their general physical condition. The wounds decreased in size and drainage diminished. The average time required for healing in the fistula patients was six months; and in the sinus group, three months.

Sixteen patients were treated with isoniazid alone. In 11 of these, the sinuses

were healed; 4 were improved; and one remained unimproved. Three patients were treated with a combination of isoniazid and para-aminosalicylic acid from the beginning. One of these was healed and 2 experienced improvement. In 8 patients, para-aminosalicylic acid was added after four months of therapy with

TABLE 2

RESULTS OF ISONIAZID OR ISONIAZID–PARA-AMINOSALICYLIC ACID TREATMENT IN 27 PATIENTS WITH TUBERCULOUS FISTULAS OR SINUSES

	NUMBER OF PATIENTS	HEALED	IMPROVED	UNIMPROVED
Bronchocutaneous fistula	15	9	5	1 (Died)
Pleurocutaneous sinus	11	9	1	1
Lymph node sinus	1	0	1	0
Total	27	18	7	2

TABLE 3

EVALUATION OF THE EFFECT ON HEALING OF TUBERCULOUS FISTULAS OR SINUSES BY THE ADDITION OF PARA-AMINOSALICYLIC ACID TO ISONIAZID

DRUG	TOTAL NUMBER	HEALED	IMPROVED	UNIMPROVED
Isoniazid alone	16	11	4	1
Isoniazid and para-aminosalicylic acid	3	1	2	0
Isoniazid followed by the addition of para-aminosalicylic acid	8	6	1	1
Total	27	18	7	2

TABLE 4

RELATION OF STREPTOMYCIN SUSCEPTIBILITY OF PATIENTS' TUBERCLE BACILLI TO RESULT OBTAINED FOLLOWING ISONIAZID THERAPY

TUBERCLE BACILLI FROM PATIENT	SINUS OR FISTULA	
	HEALED	UNHEALED
Streptomycin-resistant	6	5
Streptomycin-susceptible	5	2
Drug susceptibility undetermined	7	1
Total	18	8

One early death not included.

isoniazid. Complete healing had not occurred with isoniazid alone. After the addition of the second drug, 2 were healed, one was improved, and one remained unimproved (table 3).

An analysis of the data with respect to streptomycin resistance was made (table 4). In 11 patients, the tubercle bacilli excreted were resistant to at least

10 γ of streptomycin per ml. of medium; in 7 patients, the tubercle bacilli were susceptible to streptomycin, and in 8 patients the drug susceptibilities of their bacilli were unknown because of failure to obtain growth of the bacilli on cultures. Complete healing of the sinuses after isoniazid occurred in 6 of the group with streptomycin-resistant infections, in 5 with streptomycin-susceptible infections, and in 7 with infections of undetermined streptomycin susceptibility.

The following three case reports illustrate grave therapeutic problems which have apparently been solved by the use of isoniazid.

Case Reports

Case 1. C. D., a 36-year-old white male, was diagnosed as having pulmonary tuberculosis in 1942. A left eight-rib thoracoplasty was successfully performed for a large upper lobe cavity. He was considered to be well until 1947, when a cavity 6 cm. in diameter developed in the upper lobe of the right lung. This was treated unsuccessfully with streptomycin and concomitant pneumoperitoneum. In 1949, a six-rib thoracoplasty, accompanied by a Monaldi type of drainage, was performed on the right. A large cavity persisted under the thoracoplasty, and, on April 2, 1951, an open cavernostomy was done from a posterior approach. The thin-walled roof of this 6 cm. oval cavity was removed, leaving a large open subscapular pocket in the base of which several bronchial communications could be seen. The patient was treated with 1 gm. of streptomycin twice weekly and 12 gm. of PAS daily from December, 1950, to April, 1952. Tubercle bacilli from the drainage material were resistant to 100 γ of streptomycin per ml. of medium. The large open wound had contracted only slightly during this period. Isoniazid therapy with a daily dose of 150 mg. was begun on April 20, 1952. Improvement was immediate and progressive, and three months later the sinus was completely healed. There was a weight gain of 22 pounds and, on February 6, 1953, he was discharged from the hospital as having obtained the maximum possible benefit from therapy.

Case 2. C. M., a 38-year-old white male, was diagnosed as having pulmonary tuberculosis in 1948 on admission to Oteen Veterans Administration hospital. He was treated with streptomycin, 1 gm. daily, for forty-five days in 1948, and received pneumoperitoneum. A cavity in the right upper lobe persisted and, in 1949, a ninety-day course of streptomycin was given. A right thoracoplasty, followed by an open anterior cavernostomy, was performed in another hospital in 1949. On February 6, 1951, a right pneumonectomy was done. This was followed by a bronchopleurocutaneous fistula which was treated by further stages of thoracoplasty and an open thoracotomy. On July 20, 1951, an esophago-pleurocutaneous fistula was recognized, and a temporary jejunostomy was done for feeding. A limited Schede type of procedure was performed on August 16, 1951. Streptomycin, 1 gm. twice weekly, and PAS, 12 gm. daily, were given from May 10, 1951, to May 9, 1952. At that time the patient's tubercle bacilli were resistant to 100 γ of streptomycin and 10 γ of PAS per ml. of medium. The fistula remained open. On May 30, 1952, isoniazid, 150 mg. daily, was begun and was continued to October 10, 1952. After three months of isoniazid treatment, the fistula healed and the patient was on a normal diet. He was discharged on October 10, 1952, as having obtained the maximum benefit from hospitalization, and he has remained well. An esophagobroncho-pleurocutaneous fistula in a patient with an infection resistant to streptomycin and PAS represented a truly formidable treatment problem.

Case 3. H. C., a white female, thirty-seven years of age, was diagnosed as having pulmonary tuberculosis in 1936. She was treated with right pneumothorax from December, 1936, to May, 1946. A left pneumothorax was initiated in June, 1940, and discontinued in May, 1948, when a left pyothorax developed. This was treated by repeated thoracenteses until

September, 1949. On November 10, 1949, the left lung was decorticated in an attempt to eliminate the empyema space. The lung re-expanded but a pleurocutaneous sinus persisted which did not heal until the chest wall sinus was excised on April 18, 1950. In May, 1951, there was a recurrence of sputum positive for tubercle bacilli and a small empyema pocket was noted over the left upper lobe. A five-rib thoracoplasty was done, at which time the empyema cavity was entered and the gangrenous outer wall of a large upper lobe cavity was removed. The patient was treated for eight months with dihydrostreptomycin and PAS. At the end of that course of therapy, resistance to both of these drugs was present in tubercle bacilli obtained by culture. On March 2, 1952, there had been an exacerbation of disease in the patient's right lung and an accumulation of fluid in a large emphysematous bulla residual from her expanded right pneumothorax. The temperature ranged from 102° to 103°F. daily, and her relatives were told that her life expectancy was not more than thirty days. She was then given a short course of pyrazinamide but was unable to tolerate this drug. On February 20, 1952, 150 mg. of isoniazid were given, and this daily dosage was continued to February 13, 1953. At the start of isoniazid therapy there were three fistulous openings in the posterior thoracoplasty scar and a large axillary cavernostomy opening. Within four days after beginning isoniazid therapy, the temperature was reduced to 98.6°F. and remained at that level. Improvement in the right lung was gradual and progressive so that in four months the fluid had cleared and the right exacerbation had stabilized. The sinuses showed slow but continuous healing and were completely healed eight months after treatment was begun. The sputum became negative for tubercle bacilli two months after initiation of isoniazid therapy and has remained so during the ensuing ten months.

These 3 patients had post-surgical complications of tuberculosis which presented an immediate threat to their lives. Tubercle bacilli cultures from each patient were known to be resistant to streptomycin. Shortly after initiation of therapy with isoniazid, improvement in clinical condition and diminution of the drainage from the sinuses were apparent in all 3 patients. This improvement was progressive and continuous until complete healing was obtained.

SUMMARY

Isoniazid has proved to be an effective agent in the treatment of tuberculous sinuses and fistulas. The results following its use have been outstanding in patients whose tubercle bacilli are susceptible to streptomycin. A majority of the patients with streptomycin-resistant tubercle bacilli have also been benefited by treatment with isoniazid. The latter group represents extreme treatment failures and offers a severe test for the effectiveness of the drug. As is the case with streptomycin, all necessary auxiliary surgical procedures must be carried out concomitantly with isoniazid therapy.

SUMARIO

La Isoniacida y la Isoniacida—PAS en el Tratamiento de las Fístulas Tuberculosas

La isoniacida ha resultado ser un agente eficaz en el tratamiento de las fístulas tuberculosas. Los resultados consecutivos a su empleo han sido notables en los enfermos cuyos microbios eran sensibles a la estreptomicina. También se han beneficiado con ella la mayoría de los sujetos que tenían bacilos tuberculosos estreptomicino-resistentes. Este último grupo representa los fracasos supremos del tratamiento y ofrecen una prueba rigurosa de la eficacia de la droga. Lo mismo que con la estreptomicina, junto con la farmacoterapia, hay que llevar a cabo todas las necesarias medidas quirúrgicas auxiliares.

RESUME

L'isoniazide et l'isoniazide—PAS dans le traitement des fistules tuberculeuses

L'isoniazide a donné des preuves de son efficacité dans le traitement des abcès tuberculeux fistulisés. Les résultats ont été remarquables chez les malades dont les bacilles étaient sensibles à la streptomycine. La plupart des malades dont les bacilles étaient streptomycinorésistants ont été également améliorés. C'est dans ce dernier groupe que s'observent les échecs du traitement; celui-ci permet également les tests les plus rigoureux de l'efficacité de ce produit. De même qu'avec la streptomycine, tous les procédés chirurgicaux auxiliaires peuvent être effectués au cours du traitement par l'isoniazide.

REFERENCES

(1) Brock, B. L.: Streptomycin in treatment of draining tuberculous sinuses, J.A.M.A., 1947, *135*, 147.

(2) Bosworth, D. M., Della Pietra, A., and Farrell, R. F.: Streptomycin in tuberculous bone and joint lesions with mixed infection and sinuses, J. Bone & Joint Surg., 1950, *32-A*, 103.

(3) Murphy, J. D., and Takaro, T.: Streptomycin in the treatment of draining tuberculous sinuses: Report of a five-year follow-up, Am. Surgeon, 1952, *18*, 1057.

(4) Robitzek, E. H., and Selikoff, I. J.: Hydrazine derivatives of isonicotinic acid (Rimifon, Marsilid) in the treatment of active progressive caseous pneumonic tuberculosis: A preliminary report, Am. Rev. Tubere., 1952, *65*, 402.

THE EFFECTS OF AN INTERMITTENT DOSAGE SCHEDULE OF VIOMYCIN ON RENAL FUNCTION AND ON PLASMA ELECTROLYTES[1]

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INTRODUCTION

Since the preliminary reports (1, 2) that viomycin[2] possessed antituberculous activity against both streptomycin-susceptible and streptomycin-resistant strains of *M. tuberculosis*, numerous clinical investigators have evaluated the therapeutic merits of this agent on the tuberculous process in humans (3). During the original studies, evidence of renal toxicity appeared. For example, Werner and his associates (4) treated ten patients who had far advanced pulmonary tuberculosis with viomycin for a period up to 182 days, using 30 to 75 mg. per kg. of body weight per day. On this dosage schedule, evidence of renal toxicity was found in all patients. In addition, five patients developed abnormalities of serum electrolytes which necessitated intensive replacement therapy with potassium and calcium salts. Similar laboratory observations of renal toxicity and electrolyte imbalance were reported in nearly all of the patients treated with daily doses of 1 to 4 gm. of viomycin (5).

In contrast to this situation, Pitts and his associates (6) reported on the relative freedom of toxicity when viomycin therapy was used intermittently in sixty-seven patients. These investigators found only insignificant changes in the blood electrolyte pattern, and clinical evidence of renal toxicity was of minor and transient nature. In view of this report, it seemed worth while to evaluate the renal effects of an intermittent dosage schedule.

The purpose of the present study is to present an analysis of the effects of viomycin on differential renal function studies and on the plasma electrolytes of 14 patients who received intermittent viomycin therapy in doses of 2 gm. twice weekly by the intramuscular route.

MATERIAL AND METHODS

Fourteen patients were given viomycin intramuscularly in doses of 2 gm. twice a week. Seven patients received the drug for three to four months, and the remaining 7 patients received it for six months. All patients had far advanced pulmonary tuberculosis at the onset of therapy, and the sputum was positive on concentra-

[1] From the Departments of Medicine and Pharmacology, Baylor University College of Medicine; the Houston Tuberculosis Hospital; and the Research Division of the Veterans Administration Hospital, Houston, Texas.

[2] Supplied through the courtesy of Chas. Pfizer & Co., Inc., Brooklyn, New York, and Parke, Davis & Company, Detroit, Michigan.

tion and culture for tubercle bacilli. None of the patients had overt evidence of renal tuberculosis. The ages of the patients ranged from twenty-four to seventy-one years (*see* table 2).

Clinical laboratory studies for renal toxicity consisted of weekly urinalyses and blood urea nitrogen determinations. Creatinine clearances were done every two weeks, and phenolsulfonphthalein (PSP) clearances were done at three-month intervals. The plasma concentrations of sodium, potassium, calcium, phosphorous, chloride, and carbon dioxide combining power were also determined at weekly intervals. A Beckman flame photometer was used for sodium and potassium analyses. Carbon dioxide was determined by the Van Slyke technique.

Differential renal function studies (clearance techniques) were done on 10 patients. Following the control observations, the drug was started and the studies were repeated after three months of therapy in all of the patients and again after six months in 5 of them. Therapy was discontinued after three months in 5 of the 10 patients.

Renal clearances were determined according to the methods of Goldring and Chasis (7). Glomerular filtration rate (GFR) (inulin and endogenous creatinine clearances), renal plasma flow (RPF) (para-aminohippurate clearance), hematocrit, and urine flow were measured. Infusion of inulin and *p*-aminohippurate (PAH) into an arm vein was maintained at a constant rate by using a mercury pressure displacement pump (8). At least thirty minutes were allowed for equilibration following the priming infusion before the study was begun. Urine was collected through an indwelling Foley catheter at the end of each period by washing the urinary bladder with two successive 50 cc. portions of distilled water and 100 cc. of air. Each collection period was usually of ten minutes' duration, and blood was drawn into heparinized syringes for chemical analysis two minutes before the midpoint of each period. Para-aminohippurate was analyzed by the method of Smith (9), creatinine by the method of Bonsnes (10), and inulin by the method of Roe (11). Blood pressure was measured with a sphygmomanometer.

RESULTS

Urinalysis: The only evidence of renal toxicity in the clinical laboratory tests occurred in 5 patients, and these abnormal urinary findings are presented in table 1. The abnormalities were in the form of albuminuria and/or granular casts. Three patients with albuminuria during the control studies excreted greater or equal amounts of albuminuria at varying intervals during therapy. In 2 of these 3 patients, granular casts which were not observed during the control studies were found during viomycin therapy. However, none of them presented progressive abnormalities in plasma electrolytes (table 2). Two patients with negative urinalyses during the control periods showed evidence of albuminuria during therapy. Granular casts were observed concurrently in the urine of both of these patients. There were no instances in which these abnormalities showed progression. The urine became normal upon cessation of therapy in all instances except one, and this patient had 2-plus albuminuria and casts in the urine prior to receiving viomycin.

The fact that the abnormalities observed on urinalysis did not show progression on continued therapy and were not associated with serious abnormalities in the differential renal function studies in the patients thus studied suggests that they were not of serious consequence and may have been unrelated to the administration of viomycin. There was no evidence of altered blood urea nitrogen, PSP clearance, or in creatinine clearances.

TABLE 1

Results of Urinalyses in Five Patients During Viomycin Administration

Patient	Urinalysis*																			
	Specific Gravity					pH					Albuminuria					Microscopic†				
	C	D_1	D_2	D_3	A	C	D_1	D_2	D_3	A	C	D_1	D_2	D_3	A	C	D_1	D_2	D_3	A
1. L. A.	1,006	1,010	1,010	1,008	1,015	Alkaline	Acid	Acid	Acid	Alkaline	1+	3+	1+	1+	1+	Negative	Negative	Negative	Negative	Negative
2. J. W.	1,007	1,005	1,003	1,002	1,004	Acid	Acid	Acid	Acid	Acid	Negative	1+	Trace	Negative	Negative	Negative	Many fine granular casts	Rare fine granular casts	Negative	Negative
4. E. P.	1,026	1,013	1,012	1,024	1,020	Acid	Acid	Acid	Acid	Acid	Trace	1+	1+	1+	Negative	Negative	3 to 5 coarse granular casts	Few coarse granular casts	Few fine granular casts	Negative
6. E. S.	1,015	1,020	1,018	—	1,012	Acid	Acid	Acid	—	Acid	Negative	Trace	Trace	—	Negative	Negative	Many coarse granular casts	0	—	Negative
7. W. F.	1,015	1,014	1,012	—	1,010	Acid	Acid	Acid	—	Alkaline	2+	1+	2+	—	2+	15 to 20 leukocytes, 3 to 5 granular casts	6 to 8 leukocytes, 2 to 5 erythrocytes, 0–2 coarse granular casts	8 to 10 leukocytes, many fine and coarse granular casts	—	20 to 25 erythrocytes, occasional fine granular casts

* Random collection of urine.

† Centrifuged specimen—examined under a low-power field.

C —control observation.

D_1—After receiving viomycin for one month.

D_2—After receiving viomycin for three months.

D_3—After receiving viomycin for six months.

A —Viomycin discontinued for one month.

TABLE 2

CONCENTRATION OF SERUM ELECTROLYTES DURING VIOMYCIN THERAPY

PATIENT	AGE	SEX	SERUM SODIUM†				SERUM POTASSIUM†				SERUM CALCIUM†				SERUM PHOSPHORUS†				SERUM CHLORIDE†			
			C	D_1	D_2	D_3	C	D_1	D_2	D_3	C	D_1	D_2	D_3	C	D_1	D_2	D_3	C	D_1	D_2	D_3
1. L. A.*	71	F	134	128	135	145	4.5	4.7	4.3	4.8	5.9	5.4	5.0	4.9	1.6	1.9	1.8	2.0	98	99	97	99
2. J. W.*	45	M	144	140	143	140	5.0	4.7	4.2	4.8	4.6	4.7	5.3	5.3	1.7	2.4	1.8	1.7	104	98	98	98
3. J. B.*	66	M	142	145	148	146	4.6	4.4	4.4	4.1	4.8	5.6	5.3	5.4	1.4	1.7	1.3	1.6	111	100	104	109
4. E. P.*	41	M	137	136	133	140	5.1	5.0	4.4	4.5	5.5	5.4	5.4	5.9	2.3	2.0	3.8	1.8	103	98	102	105
5. C. J.*	42	M	146	137	138	141	5.5	5.2	4.6	5.0	5.1	5.3	5.3	5.5	1.8	1.5	1.8	1.7	104	103	103	104
6. E. S.	55	M	140	135	137	—	4.8	5.3	4.4	—	5.8	6.1	6.4	—	2.4	2.1	1.7	—	103	100	99	—
7. W. F.	31	M	144	131	141	—	4.9	4.7	3.7	—	5.1	5.4	4.9	—	1.8	1.8	1.5	—	97	98	98	—
8. M. M.	42	F	143	145	138	—	4.4	4.2	4.1	—	4.7	5.9	4.6	—	1.3	1.3	1.5	—	109	99	103	—
9. R. B.*	66	M	148	139	139	148	4.8	4.8	4.5	5.0	5.7	5.1	5.3	5.4	1.8	2.3	2.3	2.0	95	101	104	104
10. J. A.	36	F	145	141	142	—	4.2	3.2	3.4	—	5.4	5.6	5.7	—	2.1	1.8	1.4	—	95	100	99	—
11. B. W.*	55	M	135	135	134	134	4.5	4.4	4.2	4.2	4.5	4.8	4.9	4.3	2.3	2.2	2.6	2.5	101	102	99	99
12. J. A.	32	M	142	147	135	—	4.2	4.3	4.7	—	4.9	5.7	5.5	—	1.1	1.9	1.7	—	87	102	96	—
13. J. P.	70	M	139	139	138	—	4.0	4.4	4.0	—	4.8	5.2	4.7	—	2.3	2.4	2.0	—	104	96	88	—
14. D. Y.	24	M	145	138	140	—	3.8	3.8	3.6	—	4.6	4.8	5.0	—	2.0	1.8	1.9	—	98	98	101	—
Mean	48		142	138	139	142	4.6	4.5	4.2	4.6	5.1	5.4	5.2	5.2	1.9	1.9	1.9	1.9	101	100	99	101

* These patients were treated for six months.

† Milliequivalents per liter.

C—Control.

D_1–D_3—See table 1 for key to abbreviations.

Normal values (ranges for this laboratory): sodium—135 to 148 mEq. per L.; potassium—3.6 to 6.2 mEq. per L.; calcium—4.5 to 6.0 mEq. per L.; phosphorus—1.32 to 2.30 mEq. per L.; chloride (NaCl)—99 to 108 mEq. per L.

TABLE 3

EFFECT OF VIOMYCIN ON RENAL FUNCTION (CLEARANCES)*

Absolute Values

PATIENT	GLOMERULAR FILTRATION RATE, CC. PER MINUTE			CREATININE CLEARANCE, CC. PER MINUTE			PLASMA CREATININE†			RENAL PLASMA FLOW, CC. PER MINUTE			HEMATOCRIT			RENAL BLOOD FLOW, CC. PER MINUTE		
	C	D_2	D_3	C	D_2	D_3	C	D_2	D_3	C	D_2	D_3	C	D_2	D_3	C	D_2	D_3
1. L. A.	127	112	109	117	93	103	0.68	0.78	1.3	555	419	509	38	38	31	895	676	738
3. J. B.	132	120	128	101	129	98	1.44	0.78	1.2	560	540	545	30	36	32	800	844	801
4. E. P.	81	76	80	73	69	70	1.19	1.32	1.0	474	678	459	43	32	39	832	997	752
5. G. J.	121	118	102	130	91	88	0.85	1.16	1.1	652	766	502	47	46	46	1,230	1,419	1,096
6. E. S.	123	118	—	134	93	—	0.62	1.00	—	734	614	—	38	38	—	1,184	990	—
10. J. A.	110	96	—	92	108	—	0.85	0.28	—	479	553	—	48	51	—	921	1,129	—
11. B. W.	74	67	64	72	61	50	1.40	1.50	1.6	519	526	517	39	40	38	851	877	834
12. J. A.	108	99	—	77	88	—	1.40	1.60	—	600	964	—	44	43	—	1,071	1,691	—
13. J. P.	81	74	—	81	75	—	1.20	1.30	—	343	484	—	38	35	—	553	745	—
14. D. Y.	111	128	—	110	138	—	1.40	0.99	—	868	927	—	42	42	—	1,497	1,598	—
Mean	108	101	97	99	94	82	1.10	1.07	1.3	578	647	524	41	40	37	983	1,097	844

* Not corrected to standard surface area. Each patient served as his own control.

† Milligrams per 100 ml.

C —Control.

D_2—After receiving viomycin for three months.

D_3—After receiving viomycin for six months.

Serum electrolytes: Observations on serum electrolytes are presented in table 2. There was little if any evidence of alteration due to viomycin. The serum sodium was depressed in a few instances, but in these cases the control values were

usually low. This was probably a reflection of the underlying disease state and not of the viomycin therapy, since, when each patient was used as his own control, these changes were not great. Asymptomatic hyponatremia has previously been reported (12) in patients with chronic tuberculosis who did not receive viomycin. Five patients showed some decrease in serum potassium (more than 0.5 mEq.) but this was never marked and tended to correct itself despite continued therapy. There were no alterations observed in carbon dioxide combining power.

TABLE 4

EFFECT OF VIOMYCIN ON RENAL FUNCTION

Expressed in Per Cent of Control Values*

PATIENT	GLOMERULAR* FILTRATION RATE, CC. PER MINUTE		CREATININE CLEARANCE, CC. PER MINUTE		RENAL PLASMA FLOW, CC. PER MINUTE		RENAL BLOOD* FLOW, CC. PER MINUTE	
	D_3	D_6	D_3	D_6	D_3	D_6	D_3	D_6
1. L. A.	82	80	75	88	75	92	76	82
3. J. B.	91	97	128	97	96	97	106	100
4. E. P.	94	99	95	96	143	97	120	90
5. G. J.	98	84	70	68	117	91	115	89
6. E. S.	96	—	69	—	84	—	84	—
10. J. A.	87	—	117	—	115	—	123	—
11. B. W.	91	86	85	69	101	100	103	98
12. J. A.	92	—	114	—	161	—	158	—
13. J. P.	91	—	93	—	141	—	135	—
14. D. Y.	115	—	125	—	107	—	107	—
Mean	94	89	97	84	114	95	113	92

* Normal day-to-day variation (expressed as per cent of control) in this laboratory is less than 18 per cent for glomerular filtration rate and 26 per cent for renal blood flow.

D_3—After receiving viomycin for three months.

D_6—After receiving viomycin for six months.

Renal clearance studies: The renal clearance (hemodynamics) studies are presented in tables 3 and 4. Only one patient showed any significant changes in glomerular filtration rate (L. A.), and this patient was underweight and showed some progression of disease during therapy. This may well explain the slight reduction in glomerular filtration rate. Although there was some variability of renal blood flow from one month to the next in some of the patients, this was no greater than one sees in random testing of normal control subjects. The blood pressure was not altered by viomycin therapy.

COMMENT

The above observations indicate that, on the intermittent low dosage schedule employed in the present study, there was no evidence that viomycin produced significant renal toxicity. Moreover, there was little, if any, effect on the plasma electrolytes, suggesting that electrolyte excretion was not altered on this regimen of viomycin therapy. Whether this freedom of toxicity in comparison with that of

previous reports is due to the dosage schedule employed or to improved methods of manufacturing the drug (decreased impurities) has not been determined. For this reason, further toxicity studies on dogs, using larger daily doses of the drug, have been undertaken and the results are to be reported subsequently.

SUMMARY

Studies on disturbances of electrolyte metabolism and on renal function studies following the administration of 2 gm. of viomycin twice weekly have been made. There was no evidence of serious disturbances of serum electrolytes or of renal function. During the study some variation in glomerular filtration rate and renal blood flow was noted, but this was no greater than would be expected on repeated testing of the same person during a six-month period.

SUMARIO

Los Efectos de un Esquema Posológico Intermitente de Viomicina sobre la Función Renal y sobre los Electrolitos del Plasma

Estos estudios tuvieron por objeto los trastornos del metabolismo de los electrolitos y de la función renal consecutivamente a la administración de 2 gm. de viomicina dos veces semanales. No se descubrieron signos de trastornos graves de los electrolitos séricos o de la función renal. Durante el estudio, notóse alguna variación de la velocidad de la filtración glomerular y de la circulación renal de la sangre, pero no mayor de lo que sería de esperar al verificar ensayos repetidos en la misma persona durante un período de seis meses.

RESUME

Effets d'un traitement intermittent avec la Viomycine sur la fonction du rein et les électrolytes du plasma.

Des recherches ont été effectuées sur les troubles du métabolisme des électrolytes et de la fonction rénale consécutifs à l'administration bi-hebdomadaire de 2 grammes de Viomycine. Aucun trouble sérieux ne s'est manifesté à l'egard des électrolytes du sérum ou de la fonction rénale. Au cours de l'étude, on a enregistré une certaine variation dans le taux de filtration glomérulaire et du débit rénal, mais ceci n'était pas plus marqué que les variations que montreraient, chez le même sujet, des tests repétés pendant une période de six mois.

REFERENCES

(1) Finlay, A. C., Hobby, G. L., Hochstein, F., Lees, T. M., Lenert, T. F., Means, J. A., P'An, S. Y., Regna, P. P., Routien, J. B., Sobin, B. A., Tate, K. B., and Kane, J. H.: Viomycin, a new antibiotic active against *Mycobacteria*, Am. Rev. Tuberc., 1951, *63*, 1.

(2) Bartz, Q. R., Ehrlich, J., Mold, J. D., Penner, M. A., and Smith, R. M.: Viomycin, a new tuberculostatic antibiotic, Am. Rev. Tuberc., 1951, *63*, 4.

(3) Third Viomycin Conference arranged by Chas. Pfizer & Co., Inc., September, 1952.

(4) Werner, C. A., Tompsett, R., Muschenheim, C., and McDermott, W.: The toxicity of viomycin in humans, Am. Rev. Tuberc., 1951, *63*, 49.

(5) Second Viomycin Conference arranged by Chas. Pfizer & Co., Inc., September, 1951.

(6) Pitts, F. W., O'Dell, E. T., Dye, W. E., and Hughes, F. J.: Intermittent viomycin therapy in pulmonary tuberculosis, Transactions of the eleventh Streptomycin Conference, Veterans Administration, 1952, p. 279.

(7) Goldring, W., and Chasis, H.: Hypertension and Hypertensive Disease, Commonwealth Fund, Division of Publication, London, 1944.

(8) Sirota, J. H., Baldwin, D. S., and Villarreal, H.: Diurial variation of renal function in man, J. Clin. Investigation, 1950, *29*, 187.

(9) Smith, H. W., Finklestein, N., Alminosa, L., Crawford, B., and Graber, M.: The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dogs and man, J. Clin. Investigation, 1945, *158*, 581.

(10) Bonsnes, R. W., and Taussky, H. H.: On the colorimetric determination of creatinine by the Jaffe reaction, J. Biol. Chem., 1945, *158*, 581.

(11) Roe, J. H., Epstein, J. H., and Goldstein, N. P.: A photometric method for the determination of inulin in plasma and urine, J. Biol. Chem., 1949, *178*, 839.

(12) Sims, E. A., Welt, L. G., Orloff, J., and Needham, J. W.: Asymptomatic hyponatremia in pulmonary tuberculosis, J. Clin. Investigation, 1950, *29*, 1545.

VIRULENCE OF TUBERCLE BACILLI RECOVERED FROM PATIENTS TREATED WITH ISONIAZID[1, 2, 3]

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INTRODUCTION

During an investigation of the practical significance of the isolation of tubercle bacilli resistant to isoniazid *in vitro*, guinea pigs were infected with cultures exhibiting various degrees of resistance and were subsequently treated with isoniazid. These experiments were similar in type to those previously reported from this laboratory in studies of streptomycin (1). In May of 1952, guinea pigs were infected with the H37Rv strain of *M. tuberculosis* which had been made highly resistant to isoniazid by serial transfer in drug-containing liquid Tween[4]-albumin medium. This strain had undergone ten transfers at two- to three-week intervals and was able to grow in at least 50 γ of isoniazid per ml. Although its growth characteristics and colonial morphology on solid and liquid media seemed to be identical to the parent strain, it was found that the drug-resistant organisms were not virulent for guinea pigs. The same strain was retested after six transfers in liquid medium without drugs (during which drug resistance was maintained), and again was found to be nonvirulent for guinea pigs. This observation stimulated interest in the further study of the virulence of strains of tubercle bacilli isolated from patients' sputum before and during treatment with isoniazid, as well as the virulence of the organisms in the sputum before isolation on artificial media.

It is the purpose of this report to present the early results of this study, realizing that much more work needs to be done before the true significance of the data can be evaluated, both bacteriologically and clinically.

MATERIALS AND METHODS

Guinea pigs were infected subcutaneously in the inguinal region with either a suspension of tubercle bacilli or the digested and concentrated sputum. The organisms were picked directly from the primary isolation on solid egg-yolk–potato-glycerin medium and a heavy suspension made in physiologic saline. The inoculum was estimated to contain approximately 0.1 mg. dry weight of bacilli. Each specimen was inoculated into a group of either 2 or 5 guinea pigs. The virulence of the tubercle bacilli was judged by the number of deaths from tuberculosis and the amount of disease observed when the surviving animals were

[1] From the Trudeau Laboratory of The Trudeau Foundation for Clinical and Experimental Study of Pulmonary Disease, Trudeau, New York.

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[3] Preliminary report presented at the Twelfth Veterans Administration-Army-Navy Conference on the Chemotherapy of Tuberculosis held in Atlanta, February, 1953.

[4] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

killed approximately 100 days after infection. The virulence was graded according to the following scale:

Good—generalized disease of the lungs, liver, spleen, and lymph nodes, and at least one death from tuberculosis in the group.

Fair—gross disease of the internal organs but less extensive than in the previous category, and no deaths from tuberculosis.

Slight—a local abscess at the site of inoculation with caseation of the inguinal and iliac lymph nodes, but no more than an occasional tubercle in the liver or spleen.

None—no evidence of tuberculosis anywhere in the body or, at the most, a small local abscess with no involvement of the regional lymph nodes.

The isoniazid susceptibility of each culture was determined by titration in solid medium containing graded concentrations of the drug. Degrees of susceptibility were defined as follows:

Susceptible—no appreciable growth in solid medium containing 0.2 γ of isoniazid per ml; *slightly resistant*—growth equal to or only slightly less than the control tube in medium containing 0.2 γ of isoniazid, but no appreciable growth in medium containing 1.0 γ of isoniazid per ml; *moderately resistant*—growth equal to or only slightly less than the control in medium containing 0.2 and 1.0 γ of isoniazid per ml; *highly resistant*—growth equal to or only slightly less than the control in medium containing 1.0 γ of isoniazid per ml, and an appreciable amount of growth in 10 γ per ml.

RESULTS

Since there was no appreciable difference in the results obtained with the two different types of inoculum, culture isolates or sputum concentrates, the results obtained with both types of material will be considered together.

Strains from patients who had received no isoniazid: In table 1 may be seen the results of the virulence tests with tubercle bacilli from 8 patients with pulmonary tuberculosis who had never had isoniazid. These cultures were all isoniazid susceptible *in vitro*.

It will be noted that 4 of these strains showed good virulence, 3 showed fair virulence, and one showed only slight virulence for guinea pigs. Of the 4 which exhibited good virulence, 3 came from patients who had received no previous drug therapy, while one patient had had six weeks of streptomycin alone. Of the 3 cultures with fair virulence, 2 were obtained from patients who had received eleven to fifteen months of streptomycin and para-aminosalicylic acid (PAS) therapy and the 2 strains showed some degree of resistance to both of these drugs, while one strain came from a patient who had received no previous drug therapy. The one strain which showed slight virulence came from a patient who had been given long-term and multiple drug therapy, and whose tubercle bacilli were highly resistant to both streptomycin and PAS.

Strains obtained from patients during isoniazid therapy: In table 2 are presented the data from 25 patients during treatment with isoniazid, and the relationship of the factors of isoniazid susceptibility *in vitro*, and duration of therapy, to the virulence of the cultures. Two cultures obtained at different time intervals were obtained from 6 of these patients, and from one patient 3 separate cultures were tested.

Fourteen strains (obtained from 21 patients) demonstrated no virulence for

TABLE 1

VIRULENCE FOR GUINEA PIGS OF TUBERCLE BACILLI FROM EIGHT PATIENTS WITH PULMONARY TUBERCULOSIS—BEFORE ISONIAZID THERAPY STARTED

PATIENT	PREVIOUS THERAPY	IN VITRO SENSITIVITY OF CULTURE			FATE OF INFECTED GUINEA PIGS*		VIRULENCE‡
		Streptomycin	PAS	Isoniazid	Mortality	Average Index of Tuberculosis†	
M. W.	None	Susceptible	Susceptible	Susceptible	4 of 5	13.7	Good
I. P.	None	Susceptible	Susceptible	Susceptible	2 of 5	14.3	Good
H. H.	None	Susceptible	Susceptible	Susceptible	1 of 5	11.6	Good
A. V.	Streptomycin, 1949, 42 days	Susceptible	Susceptible	Susceptible	2 of 5	12.8	Good
F. C.	None	Susceptible	Susceptible	Susceptible	0 of 5	9.4	Fair
A. M.	2 courses of streptomycin and PAS, 4 months and 7 months	Moderately resistant	Highly resistant	Susceptible	0 of 5	10.6	Fair
M. O.	2 courses of streptomycin and PAS, 5 months and 10 months	Slightly resistant	Highly resistent	Susceptible	0 of 5	7.7	Fair
A. Q.	Streptomycin, 1948, 56 gm.; PAS, 9 months; streptomycin and PAS, 5 months; amithiosone, 6 months	Highly resistant	Highly resistant	Susceptible	0 of 5	4.8	Slight

* Survivors killed approximately 100 days after infection.

† Based on maximum of 16 for each animal: lungs, liver, spleen, and lymph nodes each assigned a value of 0 to 4, depending on amount of macroscopic tuberculous disease.

‡ See text for definition of degrees of virulence.

TABLE 2

VIRULENCE FOR GUINEA PIGS OF TUBERCLE BACILLI FROM TWENTY-FIVE PATIENTS WITH PULMONARY TUBERCULOSIS—AFTER ISONIAZID THERAPY FOR 53 TO 223 (AVERAGE, 155) DAYS. RELATION OF IN VITRO ISONIAZID SUSCEPTIBILITY AND DURATION OF THERAPY

DEGREE OF VIRULENCE*	NUMBER OF TESTS	DAYS OF ISONIAZID THERAPY WHEN SPUTUM WAS OBTAINED		IN VITRO ISONIAZID RESISTANCE OF CULTURES USED TO INFECT GUINEA PIGS*		
		Extremes	Average	High	Moderate	Slight
Good	9	128–213	179	2	4	3
Fair	5	53–221	120	0	3	2
Slight	5	97–207	160	2	3	0
None	14	62–223	173	5	9	0

* See text for definition of degrees of virulence and of drug resistance.

TABLE 3

INFLUENCE OF DURATION OF ISONIAZID TREATMENT ON VIRULENCE OF TUBERCLE BACILLI RECOVERED FROM SPUTUM

DURATION OF ISONIAZID THERAPY	NUMBER OF TESTS SHOWING VIRULENCE				TOTAL NUMBER OF TESTS
	Good	Fair	Slight	None	
d					
50–100	0	2	1	1	4
101–150	3	2	1	3	9
151–200	3	0	2	3	8
>200	3	1	1	7	12

guinea pigs, and 9 (obtained from 7 patients) showed good virulence for guinea pigs. It may be seen from table 2 that the correlation between the degree of *in vitro* isoniazid resistance and the degree of virulence for guinea pigs is not clear-cut. Six of the 9 fully virulent cultures were either moderately or highly resistant to isoniazid.

TABLE 4

VIRULENCE FOR GUINEA PIGS OF TUBERCLE BACILLI FROM PATIENTS TREATED WITH ISONIAZID. RESULTS OF REPEATED TESTS FROM THE SAME PATIENT

PATIENT	PREVIOUS CHEMOTHERAPY	DAYS OF ISONIAZID	IN VITRO ISONIAZID SUSCEPTIBILITY*	MATERIAL INOCULATED INTO GUINEA PIGS	VIRULENCE*
A. M.	2 courses of streptomycin and PAS, 4 months and 7 months	Pre-isoniazid	Susceptible	Culture	Fair
		62	Slightly resistant	Culture	Fair
		166	Moderately resistant	Culture	Slight
		223	Moderately resistant	Sputum	None
M. O.	2 courses of streptomycin and PAS, 5 months and 10 months	Pre-isoniazid	Susceptible	Culture	Fair
		53	Slightly resistant	Culture	Fair
		188	Highly resistant	Culture	Good
		188	Highly resistant	Sputum	Good
A. Q.	Streptomycin, 1948, 56 gm.; PAS, 9 months; streptomycin and PAS, 5 months; amithiozone, 6 months	Pre-isoniazid	Susceptible	Culture	Slight
		62	Moderately resistant	Culture	None
		188	Highly resistant	Culture	Slight
		188	Highly resistant	Sputum	None
M. S.	2 courses of streptomycin and PAS, 5 months and 2 months	180	Moderately resistant	Culture	Good
		213	Moderately resistant	Sputum	Good
A. L.	Streptomycin, 2 months; streptomycin and PAS, 8 months	128	Slightly resistant	Culture	Good
		181	Slightly resistant	Sputum	Good
I. R.	Streptomycin, 4 months; 2 courses of streptomycin and PAS, 5 months and 12 months	144	Moderately resistant	Culture	None
		221	Moderately resistant	Sputum	None
M. T.	Streptomycin and PAS, 9 months	133	Moderately resistant	Culture	None
		203	Highly resistant	Sputum	None

* See text for definition of degrees of virulence and of drug resistance.

Duration of isoniazid therapy: It has already been demonstrated (2–7) that there is a direct relationship between the duration of isoniazid therapy and both the percentage of patients who show isoniazid-resistant organisms *in vitro* and the degree of *in vitro* resistance. It was therefore of interest to see if there was a correlation between the degree of virulence and the duration of isoniazid therapy of the patient. It may be seen from table 2 that these two factors did not seem to be related in any way. The association of duration of therapy with degree of virulence was examined in a different manner, as shown in table 3, by dividing the 33 strains into 4 groups, depending on the days of isoniazid administration to the patient. The results of the tests were then classified ac-

cording to degree of virulence. It may be seen that the two factors appear to be independent of each other when analyzed in this fashion.

Strains isolated from same patient at different times during isoniazid therapy: The results obtained with the strains of tubercle bacilli obtained at different times from the same patient are indicated in table 4.

Pretreatment cultures were tested from only the first 3 patients. It may be seen that the cultures obtained from patient A. M. at intervals during the administration of isoniazid showed a progressive loss of virulence, whereas those obtained from patient M. O. seem to have increased slightly in virulence. In the case of patient A. Q., whose culture showed only slight virulence before treatment was started, no significant trend in the virulence was demonstrated. Cultures isolated at two different times during isoniazid therapy from patients M. S. and A. L. demonstrated a consistently good virulence, whereas those obtained from patients I. R. and M. T. were consistently nonvirulent. Data are also available from the resected lung specimen of patient I. R. after six and one-half months of isoniazid treatment. Cultures from 3 separate lesions were all positive for tubercle bacilli. Material from two of the lesions failed to produce disease in guinea pigs, and from the third produced only a local abscess at the site of inoculation with involvement of the contiguous lymph nodes but no generalized disease.

Results of tuberculin tests of inoculated guinea pigs: All of the guinea pigs involved in these experiments were subjected to intracutaneous tests with 5 per cent Old Tuberculin at least once after infection. With very few exceptions (one negative and four slightly positive reactions), the reactions were strongly positive, even in those animals which showed little tuberculous disease. The 4 animals infected with the H37Rv strain resistant to isoniazid demonstrated positive skin tests three weeks later.

Results of subcultures from infected guinea pigs: At autopsy, cultures were made from the local caseous abscesses or caseous lesions in the lymph nodes of the majority of the guinea pigs. Material from animals which had been infected with a total of 14 so-called "nonvirulent" or "slightly virulent" strains was subcultured and, with 13 of them, viable tubercle bacilli were recovered from one or more guinea pigs in the group. A total of 28 animals infected with these 14 strains was subcultured, and 22 of them yielded positive recoveries.

It is thus apparent that virtually all of these strains of tubercle bacilli, although failing to produce progressive disease in guinea pigs, did have the ability to sensitize the animals to Old Tuberculin and could be recovered from the local lesions by subculture approximately 100 days after inoculation.

All of the recovered strains were tested *in vitro* for their isoniazid susceptibility and all gave approximately the same results as the original culture used for inoculation.

Effect on virulence of continued cultivation in vitro: The loss of virulence of the H37Rv strain coincident with the development of isoniazid resistance by virtue of repeated passages in drug-containing medium could conceivably be due to the change from isoniazid susceptibility to isoniazid resistance or to the continued

passage in Tween-albumin liquid medium with or without the drug. An investigation is now in progress in which virulence for guinea pigs is determined after each transfer of the H37Rv strain in Tween-albumin medium, one substrain being carried in plain medium and the other in increased concentrations of isoniazid.

The results after ten transfers at two- to three-week intervals are indicated in table 5.

It may be seen that the virulence of the substrain carried in plain medium has remained essentially unaltered, while that of the substrain carried in increased concentrations of isoniazid showed a sharp decline at the seventh transfer. It may also be noted, however, that this change in virulence did not coincide

TABLE 5

VIRULENCE FOR GUINEA PIGS OF THE H37Rv STRAIN OF M. TUBERCULOSIS AFTER EACH PASSAGE IN TWEEN-ALBUMIN LIQUID MEDIUM

TRANSFER NUMBER	MEDIUM WITHOUT DRUG	MEDIUM WITH INCREASED CONCENTRATIONS OF ISONIAZID	HIGHEST CONCENTRATION OF ISONIAZID ALLOWING GROWTH IN 14 DAYS
			γ per ml.
1	Good	Good	0.04
2	Good	Good	0.8
3	Good	Good	5.0
4	Good	Fair	20.0
5	Good	Good	50.0
6	Good	Good	50.0
7	Fair	None	25.0
8	Good	None	25.0
9	Good	None	25.0
10	Good	None	50.0

with the onset of the emergence to predominance of drug-resistant bacilli, which had occurred at the fifth transfer. Indeed, the resistance of the substrain decreased somewhat after the sixth transfer. (The resistance level was maintained at 50 γ per ml. from the tenth to the twelfth transfer.) It would appear that the conditioning factor on which loss of virulence depends is the growth of the organisms in the presence of high concentrations of isoniazid.

DISCUSSION

Bloch and Noll (8) have described a loss of virulence for mice when the H37Rv strain was cultivated for just one generation in the presence of amithiozone or Tween 80 (the latter in much higher concentrations than the 0.02 per cent used in our Tween-albumin medium). This was accompanied by a decrease in cord formation. The present writers have also noted a loss of virulence for guinea pigs in an H37Rv culture after it had been made resistant successively to PAS, streptomycin, neomycin, and viomycin.

That not all isoniazid-resistant tubercle bacilli have lost their virulence for experimental animals is illustrated by the experiments of Karlson and Ikemi

(9), Goulding and his associates (10), and Barnett, Bushby, and Mitchison (11), who have described the production of virulent infections in guinea pigs and mice with isoniazid-resistant strains.

It is obvious from the data of the present study that the phenomenon of loss of virulence of tubercle bacilli for guinea pigs is related to other factors in addition to the development of isoniazid resistance. Some of the cultures possessing the best virulence came from patients who had received isoniazid for more than 200 days and whose tubercle bacilli showed a high degree of isoniazid resistance *in vitro*. At least one culture of only slight virulence for guinea pigs was isolated from a patient before isoniazid was started. The patient from whom the latter culture was obtained had received courses of streptomycin, PAS, and amithiozone previously. The majority of the patients had received prior chemotherapy and many of the cultures showed *in vitro* resistance to streptomycin, PAS, or both. Further work must obviously be done to determine the frequency of the occurrence of loss of virulence of tubercle bacilli from patients on treatment with all the antituberculous drugs, alone and in combination. Organisms must be recovered from such patients prior to the administration of drugs and then periodically thereafter.

Many experiments have been carried out to determine if freshly isolated strains of tubercle bacilli vary in their virulence, and if such variation can be correlated with the severity of the clinical disease. Pätiälä (12) gives an excellent summary of the literature on this subject, and two other papers on this question (13, 14) have recently appeared. The results have been contradictory, but the majority of studies have indicated that strains isolated from different patients do vary in their ability to produce disease in animals. The correlation of virulence for animals with clinical type of disease is not well established.

In 1948 (1) an experiment was reported from this laboratory in which guinea pigs were infected with tubercle bacilli exhibiting various degrees of *in vitro* streptomycin resistance isolated from patients treated with streptomycin alone. None of these strains showed loss of virulence. Efforts are now being made in many laboratories to study the viability and virulence of tubercle bacilli from resected lung lesions of man. These studies have shown that, in many instances, especially after prolonged chemotherapy, it is impossible to grow the bacilli or produce disease in guinea pigs or mice although very many stainable tubercle bacilli may be seen on smear. This is a phenomenon which may be related to the one under discussion. It differs, however, in that these stainable organisms will not grow on culture, whereas the strains described in the present paper all grow readily on artificial media.

Although the early findings described in this paper are of interest and should add to the knowledge of what constitutes virulence of tubercle bacilli, the possibly erroneous interpretation should be avoided that these tubercle bacilli are now harmless for those who harbor them as well as for others who may become infected with them. A preliminary survey of the correlation between virulence of the culture and the further course of the patient's tuberculosis indicates that *patients whose bacilli demonstrate very slight or no virulence for guinea pigs may nevertheless undergo serious exacerbations of disease.*

Investigation is urgently required along the lines of: large-scale study of the virulence of the cultures of tubercle bacilli from patients before, during, and after various chemotherapy regimens; the correlation between the virulence of cultures and the clinical course of the patient harboring the bacilli; the permanence of the loss of virulence for laboratory animals after the patient is no longer taking the drug; the nature of the mechanisms responsible for the alteration in virulence of the tubercle bacilli, including investigation of all the known factors conditioning the virulence of tubercle bacilli; and the determination of any possible alteration in the host in regard to susceptibility to these changed tubercle bacilli.

SUMMARY

A strain of *M. tuberculosis* (H37Rv) lost its virulence for guinea pigs after it was made resistant to isoniazid by serial transfer in liquid medium containing increased concentrations of the drug. The same phenomenon has been observed previously after the H37Rv strain was made resistant in a similar manner to a series of four drugs successively.

Tubercle bacilli isolated from certain patients under treatment with isoniazid have also demonstrated a loss of virulence for guinea pigs. This loss of virulence was not correlated with the duration of isoniazid therapy or with the degree of *in vitro* isoniazid resistance.

At least one patient had tubercle bacilli with little virulence for guinea pigs even before isoniazid was started.

Much further work remains to be done before the true significance of these findings can be determined.

SUMARIO

Virulencia de los Bacilos Tuberculosos Obtenidos de Enfermos Tratados con Isoniacida

Una cepa H37Rv de bacilos tuberculosos perdió su virulencia para los cobayos después de convertirla en resistente a la isoniacida mediante pases seriados por un medio líquido que contenía concentraciones cada vez mayores de la droga. El mismo fenómeno había sido observado antes, después de convertir la cepa H37Rv en resistente en forma semejante a una serie de cuatro drogas sucesivamente.

Los bacilos tuberculosos aislados de ciertos enfermos en vías de tratamiento con isoniacida también han mostrado pérdida de virulencia para los cobayos. Esa pérdida de virulencia no se correlacionó con la duración de la isoniacidoterapia ni con la intensidad de la isoniacidorresistencia *in vitro*.

Aun antes de iniciar la isoniacida, a lo menos un enfermo tenía bacilos tuberculosos dotados de poca virulencia para el cobayo.

Hay que realizar todavía mucha investigación más antes de poder apreciar la verdadera importancia de esos hallazgos.

RESUME

Virulence des bacilles tuberculeux récupérés chez des malades traités par l'Isoniazide

Une souche H37Rv de bacilles tuberculeux a perdu sa virulence pour le cobaye après qu'elle ait été rendu résistante à l'isoniazide par le transfert en série dans un milieu liquide contenant des concentrations croissantes d'isoniazide. Le même phénomène a été observé antérieurement après que, par le même procédé, la souche H37Rv eût été rendu résistante à une série de quatre substances expérimentées successivement.

Chez le cobaye, les bacilles tuberculeux isolés chez certains malades, soumis au traitement par l'isoniazide, ont présenté une perte de virulence. Il n'y avait aucune corrélation entre cette perte de virulence et la durée du traitement par l'isoniazide ou le degré de la résistance à l'isoniazide *in vitro*.

Parmi les cas observés, au moins un d'entre eux avait des bacilles tuberculeux dont la virulence pour le cobaye était faible, dès avant l'instauration du traitement par l'isoniazide.

D'autres études devront être entreprises avant que la véritable signification de ces résultats puisse être précisée.

REFERENCES

(1) Steenken, W., Jr., and Wolinsky, E.: Streptomycin in experimental tuberculosis: Response in guinea pigs infected with strains of varying degrees of streptomycin resistance, Am. Rev. Tuberc., 1948, *58*, 353.

(2) Steenken, W., Jr., and Wolinsky, E.: Isoniazid in experimental tuberculosis, Nat. Tuberc. A. Tr., 1952, p. 425.

(3) Medical Research Council: Tuberculosis Chemotherapy Trials Committee: Isoniazid in the treatment of pulmonary tuberculosis, Brit. M. J., 1953, No. 4809, 521.

(4) Dye, W. E., Lynch, H. P., and Brees, A. G.: Incidence of bacterial resistance encountered with tuberculosis chemotherapy regimens employing isoniazid alone and in combination with intermittent streptomycin, Am. Rev. Tuberc., 1953, *67*, 106.

(5) Thoren, M., and Hinshaw, H. C.: Therapy of pulmonary tuberculosis with isoniazid alone and in combination with streptomycin and with para-amino-salicylic acid, Stanford M. Bull., 1952, *10*, 316.

(6) Petit, A.: Preliminary note on appearance of resistance to isonicotinic acid hydrazide, Scalpel, 1952, *105*, 1131.

(7) Ferebee, S. H.: The effect of streptomycin on the emergence of bacterial resistance to isoniazid: A United States Public Health Service cooperative investigation, Am. Rev. Tuberc., 1953, *67*, 553.

(8) Bloch, H., and Noll, H.: Studies on the virulence of tubercle bacilli: Variations in virulence effected by Tween 80 and thiosemicarbazone, J. Exper. Med., 1953, *97*, 1.

(9) Karlson, A. G., and Ikemi, Y.: Effect of isonicotinic acid hydrazide (isoniazid) on survival time of mice infected with tubercle bacilli resistant to the drug *in vitro*, Proc. Staff Meet., Mayo Clin., 1952, *27*, 373.

(10) Goulding, R., King, M. B., Knox, R., and Robson, J. M.: Relation between *in vitro* and *in vivo* resistance to isoniazid, Lancet, 1952, *2*, 69.

(11) Barnett, M., Bushby, S. R. M., and Mitchison, D. A.: Isoniazid-resistant strains of tubercle bacilli: Their development and stability, Lancet, 1953, *1*, 314.

(12) Pätiälä, J.: On different strains of tubercle bacilli in their relation to the clinical picture of the tuberculosis caused by them, Acta tuberc. Scandinav., 1948, *22*, 227.

(13) Dhayagude, R. G., and Shah, B. R.: Variation in the virulence of *M. tuberculosis* and its correlation with the clinical type of tubercular disease, Indian J. M. Research, 1948, *36*, 79.

(14) Bergeron, A., and Lavagne-Mézière: Relations entre les virulences des bacilles de Koch et les formes cliniques de la tuberculose pulmonaire, Rev. de la tuberc., 1950, *14*, 694.

CERTAIN NONPATHOGENIC MYCOBACTERIA[1]

As a Source of Error in Diagnosis and Drug-Susceptibility Tests

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INTRODUCTION

The present extensive use of chemotherapy in tuberculosis has encouraged the bacteriologist to expend greater effort in culturing tubercle bacilli from pathologic material. This procedure is essential to the performance of the drug-susceptibility tests which are carried out before, during, and after chemotherapy to aid in the selection of the drugs to be administered and in the evaluation of therapy. The renewed interest in the isolation of tubercle bacilli has resulted in an awareness of the limitations of the cultural methods and of certain pitfalls which may be encountered.

Recently, the present writers have noted the occasional primary recovery of *Mycobacteria* which, although nonpathogenic for the guinea pig, are grossly indistinguishable from colonies of *Mycobacterium tuberculosis*. Such a situation may result in a false bacteriologic diagnosis of tuberculosis and has been a source of error in recording the results of drug-susceptibility studies; this group of organisms varies greatly with regard to drug susceptibility. Some strains are susceptible to streptomycin (SM) and para-aminosalicylic acid (PAS) and to a lesser degree to isoniazid; others are naturally resistant to all three drugs; and still others are streptomycin-susceptible and PAS-resistant. It is the purpose of the present communication to minimize this source of error by pointing out some observations on the detection of these acid-fast organisms and their subsequent differentiation from *M. tuberculosis*.

METHOD OF INVESTIGATION

In the present report, consideration is not given to the chromogenic acid-fast organisms since these are readily recognizable on culture. The present studies are concerned only with those bacilli whose growth characteristics are so similar to those of the tubercle bacillus that they might erroneously be mistaken for this pathogen. It would be well to point out here, however, that in some of the chromogens, especially on primary isolation, pigment appears only after the culture has been kept at 37°C. or room temperature for some time.

In this laboratory the screening test for drug susceptibility consists of inoculating five tubes of egg medium (Hohn's), each containing one of the following drug concentrations: streptomycin, 10 γ per ml.; PAS, 10 γ per ml.; and isoniazid, 5 γ per ml.; and two control tubes without any drug. This test may be performed directly from the concentrated sediment of the sputum or other material or indirectly from a suspension of the growth obtained on culture. Further susceptibility studies are carried out in a liquid medium containing higher concentrations of drugs.

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

Any unexpected result in a drug-susceptibility test should lead to the suspicion that the organism being tested may not be the tubercle bacillus. Such a suspicious finding, for example, would be the appearance of good growth in the presence of 10 γ of PAS per ml. in a culture of tubercle bacilli from a patient who received original combined streptomycin-PAS therapy for two or three months with apparently good clinical results. On original combined treatment with streptomycin and PAS, the emergence of drug-resistant strains of tubercle bacilli is usually delayed and, if drug resistance does develop after several months of treatment, it is usually to the streptomycin rather than the PAS. The appearance of a few colonies after the sputum has been negative for tubercle bacilli on culture for some time is also to be regarded with suspicion. If these irregularities are to be recognized, the laboratory worker must know the history of the patient with regard to chemotherapy, and a complete record of the past bacteriologic findings should be readily available.

When the necessity for further study is indicated, the present writers have found it helpful to observe the growth pattern (degree of "cord" formation) as a measure of virulence (1).

A loopful of the suspected culture is inoculated in liquid Tween[2]-albumin medium containing 5 per cent plasma (2). After incubation at 37°C. until good growth is obtained, a stained preparation is examined for "cord" formation. If the bacilli occur in amorphous masses (figure 1), they may safely be considered avirulent. Differentiation on the basis of the degree of "cord" formation, ranging from a tendency toward loosely packed "cords" (figure 2) to the tightly packed, serpentine "cords" of virulent tubercle bacilli (figure 3) requires corroboration by animal inoculation, particularly until experience has been gained in evaluating the growth pattern. In this laboratory, only one of many strains of mammalian tubercle bacilli studied failed to show agreement between growth pattern and virulence. This strain, a streptomycin-dependent variant derived from the virulent H37Rv strain, proved to be a good "cord-former" although it is avirulent for guinea pigs even when the animals are treated with streptomycin (3).

OBSERVATIONS

The case reports which follow are presented to illustrate some of the circumstances which led to the detection of nonpathogenic *Mycobacteria* which might have been mistaken for tubercle bacilli.

Case 1. This patient had pulmonary tuberculosis with sputum highly "positive" by smear and culture. The organisms were sensitive to 10 γ of streptomycin and 10 γ of PAS per ml. on a pretreatment direct drug-susceptibility test. After four months of combined streptomycin-PAS therapy, the sputum was negative for tubercle bacilli by smear and culture. A subsequent sputum culture yielded one colony of acid-fast bacilli which was recorded as *M. tuberculosis*. A drug-susceptibility test of the bacilli from this colony on solid medium showed 4-plus growth, not only on 10 γ of streptomycin and 10 γ of PAS per ml., but also on 5 γ of isoniazid per ml. Further study of these organisms in liquid Tween medium showed 4-plus growth in the following drug concentrations: streptomycin, 50 γ per ml.; PAS, 100 γ per ml.; and isoniazid, 100 γ per ml. In view of this unexpected finding, the growth pattern of the culture was studied. After ten days of incubation in liquid Tween medium containing 5 per cent human plasma, the growth was granular and the stained

[2] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

FIG. 1. Avirulent strain of *Mycobacterium*, showing nonoriented clumping of bacilli. (×400)

FIG. 2. A strain of low virulence, showing poorly oriented "cord" pattern with loose arrangement of bacilli. (×400)

FIG. 3. H37Rv. Virulent human strain, showing characteristic serpentine growth. (×400)

preparation showed no tendency toward "cord" formation (figure 1). A guinea pig inoculated in the groin with a small number of these organisms became tuberculin positive with

slight enlargement of the regional lymph nodes after one month. When the animal was sacrificed two months after inoculation, no gross lesions were seen and cultures of the tissues were negative for *M. tuberculosis*.

Case 2. A patient with pulmonary tuberculosis had sputum "positive" on smear and culture. The tubercle bacilli were susceptible to streptomycin and PAS prior to the institution of combined treatment with these two drugs. The sputum was "converted" after three months of chemotherapy and then, after several negative cultures, two colonies of acid-fast bacilli were obtained. The drug susceptibility of these two colonies was tested individually and each was found to be highly resistant to PAS but susceptible to streptomycin. On further study these organisms revealed some tendency toward "cord" formation unlike that of highly virulent tubercle bacilli but indicative of some degree of virulence. Guinea pig inoculation resulted in the formation of a local abscess which gradually subsided; and, at necropsy, after three months, acid-fast bacilli could not be recovered from the abscess pus or the tissues.

Case 3. This patient had pulmonary tuberculosis which was "cured" by sanatorium care only and had been well and working in the hospital for several years. A recent routine sputum culture yielded two colonies growing approximately 3 mm. apart. One of these was dry and rough and the other was rather smooth and easily emulsified. Both contained acid-fast bacilli. Since the patient's sputum had been "culture-negative" for many years and because of the difference in the colonies, it was decided to perform separate drug-susceptibility studies. The smooth colony was found to be highly resistant to streptomycin, PAS, and isoniazid while the rough colony was susceptible to all three drugs. Study of the growth configuration in liquid medium and guinea pig inoculation revealed that the resistant colony was nonpathogenic while the rough colony, which formed tightly packed "cords," was *M. tuberculosis*. It might be well to point out at this time that colonies of virulent tubercle bacilli are soft and easily emulsified if the solid medium contains excess moisture or is softer than usual.

Case 4. A sputum sent to the laboratory by a patient seen in the outpatient clinic produced three colonies of acid-fast bacilli on culture. The culture was reported as being positive for tubercle bacilli and the tubes were kept for a subsequent pretreatment drug-susceptibility test in the event that the patient was admitted to the hospital. However, because of the lack of clinical or roentgenographic evidence of disease, the bacteriologic diagnosis was questioned. The organisms were found to be highly resistant to all three drugs and, although "cord" formation indicative of a degree of virulence similar to that of the BCG strains was observed, it could readily be distinguished from the tight "cords" of virulent *M. tuberculosis*. Animal inoculation resulted only in local abscess formation, and no acid-fast bacilli could be recovered from the necrotic material or the internal organs.

Similar strains of *Mycobacteria* have been detected in nine instances during the years 1951 and 1952; six of these during the latter year. The total number of sputum specimens cultured during these same periods was 3,099 and 5,698, respectively. It is conceivable that the future development of new and improved bacteriologic procedures may disclose a greater incidence of this type of acid-fast microorganism.

Not every instance of bacterial resistance discovered before or soon after the initiation of drug therapy can be explained by these nonpathogenic acid-fast strains. The medical literature now contains many reports of patients infected with drug-resistant bacilli, particularly those resistant to streptomycin (4, 5). The present writers have seen seven such cases, and contact with a streptomycin-resistant patient could be established in each.

A really unusual problem is presented, however, by the following patient's history.

Diagnosis was made in the outpatient department, and combined streptomycin-PAS therapy was begun after admission. Two sputum specimens submitted during the first week in the hospital were positive for tubercle bacilli. Drug-susceptibility tests on one of these showed the organisms to be susceptible to streptomycin and PAS. One month after the start of treatment, the sputum was negative on concentrated smear but the culture was "positive" with one colony. At the end of the second and third months of therapy, the sputum was "negative" by smear and culture. At the end of the fourth month, one colony was recovered. This colony was subcultured, and an indirect drug-susceptibility test revealed that the organisms were highly resistant to streptomycin, PAS, and isoniazid. There was no record of the patient's ever having taken isoniazid, so the culture was subjected to further study. In liquid Tween medium containing plasma, the bacilli formed long, tortuous "cords" strongly suggestive of virulent tubercle bacilli. Animal inoculation resulted in widespread tuberculosis.

These findings are difficult to interpret. The patient apparently had good results from therapy, with "negative" cultures at the end of two months. In fact, sputum cultures were again negative for tubercle bacilli at the end of the fifth and sixth months of treatment, even after this drug-resistant colony was recovered. The chance of selecting a single organism resistant to all three drugs is extremely remote even in a large bacterial population. The possibility that this resistant variant reached the sputum culture from an outside source must be considered in this instance.

The bacteriologic findings of the following case are likewise worth reporting:

A patient seen in the outpatient clinic had pleurisy with effusion, and a routine sputum examination was reported as positive for acid-fast bacilli (Gaffky IV) on concentrated smear. A direct drug-susceptibility "screening" test was performed on this specimen and, although the isoniazid, PAS, and control tubes were contaminated, the streptomycin tube showed confluent growth after two weeks of incubation. This was interpreted as a culture positive for *M. tuberculosis*. However, when the culture tube was re-examined at the end of four weeks of incubation, the growth was pigmented. Subsequent study revealed that the organism was also resistant to PAS, and fifteen colonies appeared on the medium which contained isoniazid. The organism was avirulent as evidenced by complete failure to form "cords" in liquid Tween medium containing plasma. Another specimen of sputum from this patient, cultured a few weeks later, yielded twenty-five colonies of tubercle bacilli which proved to be susceptible to all three drugs. In this case, although the patient did have tuberculosis, the acid-fast bacilli seen on smear of the first sputum examined were probably nonpathogenic chromogens.

DISCUSSION

In the preceding pages attention has been focused on the recovery from the sputum of patients of strains of *Mycobacteria* which differ from *M. tuberculosis* mainly in their inability to produce progressive disease in the guinea pig. As a result of the extensive use of chemotherapy in tuberculosis, the cultivation of tubercle bacilli from pathologic material has become an increasingly important procedure. However, the cultural appearance of these nonpathogenic organisms is so similar to that of virulent tubercle bacilli that in many instances they no doubt pass unrecognized. It has been mainly through drug-susceptibility studies that many of these strains have been detected.

Awareness of the possible presence of these nonpathogenic strains is of great importance to the bacteriologist, for, once suspected, their differentiation from tubercle bacilli becomes a relatively easy problem. Any unexpected result in a drug-susceptibility determination, particularly before or soon after the initiation of chemotherapy, should lead to further study of the culture being tested. In the present report some illustrative examples from experience in this laboratory have been cited.

The ability to produce progressive disease in the guinea pig was the criterion for pathogenicity. The "cord-forming" tendency of each strain was also studied and proved to be a reliable measure of virulence. No strain which failed to form "cords" produced disease in the guinea pig.

The origin of these nonpathogenic strains remains a matter for speculation. The literature contains many references to the saprophytic acid-fast strains, which grow rapidly and are usually pigmented, and to the chromogens, which develop more slowly (6, 7, 8). Both of these types are widely distributed in nature. Those organisms which are discussed in the present report show pigment only to the degree shown by virulent tubercle bacilli, which ranges from light cream to yellow on the usual media.

Three of the 9 patients from whom unusual strains of *Mycobacteria* were isolated had no history of receiving chemotherapy for tuberculosis, and one person had no evidence of clinical tuberculosis. The remaining patients were either receiving or had recently received drug therapy at the time the microorganisms were isolated. The fact that the majority of these strains were recovered from patients who had chemotherapy might be explained on the basis of performing more frequent cultural studies on this group of patients and on the fact that the majority of hospital patients have had drug therapy. It seems more likely that these strains of acid-fast bacilli existed long before the era of chemotherapy but escaped notice. It must be emphasized that the present writers detected these strains because of unusual findings in drug-susceptibility studies. There is no scientific evidence at this time, however, that these strains represent a variant of *M. tuberculosis* var. *hominis* occurring as a result of spontaneous mutation or brought about by the direct action of drugs on the original normal strain, although these possibilities cannot be ignored.

It is hoped that future improved bacteriologic methods will favor the detection of these nonpathogenic *Mycobacteria* which constitute a serious source of error, not only in diagnosis, but in the interpretation of drug-susceptibility studies.

SUMMARY

The occasional appearance on culture media of colonies of nonpathogenic *Mycobacteria* which cannot be distinguished by visual inspection from those of virulent tubercle bacilli is reported. Recognition of these is important because of the danger of false diagnosis and misinterpretation of drug-susceptibility studies. Two indications for further study of culture growths are: unexpected findings in studies, and the appearance of a few colonies after the sputum has been negative for tubercle bacilli by culture for some time either during or after chemotherapy.

The virulence of suspected cultures can be readily determined by study of the

growth pattern in liquid Tween medium containing 5 per cent plasma and by guinea pig inoculation in the case of those showing intermediate "cord" formation in liquid medium.

SUMARIO

Ciertas Bacterias No Patógenas, como Causa de Error en el Diagnóstico y en las Pruebas de Farmacosensibilidad

Descríbese la aparición de vez en cuando en los medios de cultivo de colonias de *Mycobacteria* no patógenas, que no pueden distinguirse por la inspección visual, de las de bacilos tuberculosos virulentos. El reconocimiento de las mismas es importante, dado el peligro que entrañan de motivar errores de diagnóstico y tergiversaciones en los estudios de farmacosensibilidad. Dos indicaciones para el estudio ulterior de las colonias en los cultivos son: (1) hallazgos inesperados en los estudios de la sensibilidad a ciertos medicamentos y (2) aparación de unas pocas colonias después que los cultivos del esputo han sido negativos por algún tiempo ya durante la quimioterapia o después de ella.

Cabe determinar fácilmente la virulencia de los cultivos sospechosos mediante el estudio del patrón del crecimiento en medio Tween líquido que contenga 5 por ciento de plasma y la inoculación en el cobayo cuando se trata de los que muestran formación intermedia de cordones en medio líquido.

RESUME

A propos de certaines mycobactéries non-pathogènes, sources d'erreur dans le diagnostic et dans les épreuves de sensibilité aux drogues

L'apparition occasionnelle dans les milieux de cultures de colonies de mycobactéries non-pathogènes, qui ne peuvent être différenciées visuellement de celles de bacilles tuberculeux virulents, est signalée. Cette identification est très importante pour éliminer le risque de faux diagnostic et d'erreurs d'interprétation dans les études de sensibilité à l'action des médicaments. La poursuite des recherches sur la croissance des cultures est indiquée dans deux cas: 1) présence de résultats inattendus dans les études sur la sensibilité à l'action des médicaments, 2) apparition de quelques colonies après que les crachats aient été négatifs pendant un certain temps au cours, ou à la suite de la chimiothérapie.

La virulence de cultures suspectes peut être rapidement déterminée par l'étude des modalités de la croissance dans le milieu Tween liquide renfermant 5% de plasma et par l'inoculation au cobaye dans le cas où les cultures présentent, en milieu liquide des formes intermediaires "en cordon."

REFERENCES

(1) Middlebrook, G., Dubos, R. J., and Pierce, C.: Virulence and morphological characteristics of mammalian tubercle bacilli, J. Exper. Med., 1947, *86*, 175.

(2) Yegian, D., and Kurung, J.: Growth pattern and virulence of tubercle bacilli, Am. Rev. Tuberc., 1952, *65*, 181.

(3) Vanderlinde, R. J., and Yegian, D.: The pathogenicity of streptomycin-dependent bacilli, Am. Rev. Tuberc., 1951, *63*, 96.

(4) Furtos, N. C., and Doane, E. A.: Transmission of streptomycin-resistant tubercle bacilli in man, J.A.M.A., 1949, *140*, 1274.

(5) Brennon, A. J., and Wichelhausen, R. H.: Streptomycin-resistant tubercle bacilli, J.A.M.A., 1949, *140*, 1275.

(6) Pinner, M.: Atypical acid-fast microorganisms: IV. Smooth growing tubercle bacilli, Am. Rev. Tuberc., 1935, *32*, 440.

(7) Tarshis, M. S., and Frisch, A. W.: Chromogenic acid-fast bacilli from human sources: I. Cultural studies, Am. Rev. Tuberc., 1952, *65*, 278.

(8) Tarshis, M. S., and Frisch, A. W.: Chromogenic acid-fast bacilli from human sources: II. Pathologic studies, Am. Rev. Tuberc., 1952, *65*, 289.

STUDIES ON THE MODE OF ACTION OF LYSOZYME ON MYCOBACTERIA[1, 2]

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INTRODUCTION

In a previous communication, Myrvik and Weiser (1) reported on a tuberculostatic substance present in the serum of rabbits following injection with heat-killed tubercle bacilli in the adjuvants of Freund (mineral oil and Falba).[5] The evidence presented indicated strongly that the substance described is lysozyme. The present communication is a report of attempts to determine the mode of action of lysozyme on the *Mycobacteria*.

The mode of action of lysozyme on *Micrococcus lysodeikticus* and *Sarcina lutea* has been reported to be enzymatic, and a substrate has been isolated from the cells which undergoes hydrolysis when treated with lysozyme (2). The minimum bacteriostatic and bactericidal levels of lysozyme for these organisms are apparently identical and have values of approximately 0.3 γ per ml. (1). By contrast, the difference between the minimum tuberculostatic and tuberculocidal levels of lysozyme for the BCG strain of *Mycobacterium tuberculosis* var. *bovis* is much greater (1, 3). For example, an appreciable killing of BCG was accomplished only with concentrations of lysozyme of 1,000 γ per ml., whereas 5 γ per ml. sufficed to produce bacteriostasis (1, 3). In view of this apparent difference in the behavior of *M. lysodeikticus* and BCG toward lysozyme, it was considered advisable to investigate the mode of action of lysozyme on the *Mycobacteria*.

Basic proteins of the protamine group are reported to possess antibacterial properties (4, 5, 6), and the proposal has been made (7) that their antimicrobial activity may be due to their capacity as bases (8, 9) to unite with the surface nucleic acid complexes of microorganisms. Since lysozyme is a basic protein, it seemed especially important to determine whether or not its tuberculostatic and tuberculocidal properties are due in any measure to its basic character, independent of its action as an enzyme.

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[5] The mineral oil used was Bayol F, obtained from Stanco Distributors, Inc., 26 West 14 Street, New York 11, New York; and the Falba from Pfaltz and Bauer, Inc., Empire State Building, New York, New York.

Rubini and his associates (10) have suggested that the capacity of synthetic lysine polypeptides to combine with and inactivate the viruses of tobacco mosaic and influenza results from ionic linkages between these basic materials and the acidic groups at the surface of the virus. They presented evidence that the stereochemical structure of the compounds, as well as the amino acid residues in the polypeptide, determines its antiviral activity. Bloom, Winters, and Watson (7) reported that ribonucleic acid (RNA) and desoxyribonucleic acid (DRNA) derived from yeast are equally effective in blocking the antibacterial action of protamine for *Bacillus subtilis*. Since the titration data on yeast nucleic acids (11, 12) indicate that fewer phosphoryl groups are present in the RNA molecule than in the DRNA molecule, the writers proposed that the blocking effect of RNA and DRNA on protamine activity does not result from simple salt linkage between the phosphoryl groups of the bacterial nucleic acids and the free amino groups of protamine. The blocking effect may depend instead upon a union permitted by complementary similarities in stereochemical structure of the basic protein and the nucleic acid complex.

According to the foregoing concept, lysozyme could likewise exert antibacterial action through its basic groups. Moreover, possible differences in the physical configuration of lysozyme and protamine could account for the contrasting broad antibacterial spectrum of protamine and the narrow antibacterial spectrum of lysozyme. Alternatively, the physical configuration of lysozyme could be such as to exclude antibacterial action by its basic groups and thus limit its antibacterial activity to its enzymatic property.

Whereas it appears to be well established that those lysozyme-susceptible species of bacteria which lyse when exposed to lysozyme are destroyed by its enzymatic action, there is less assurance that a similar mode of action operates for all of the susceptible species which do not undergo lysis.

GENERAL METHODS AND MATERIALS

Unless otherwise specified, the methods and materials enumerated below were employed throughout the investigation.

The strains of *Mycobacteria* were grown on Sauton's medium, pH 6.8, for six to eight days. Surface growth was removed, ground in a sterile mortar, and the cells were suspended in sterile physiologic saline containing 0.04 per cent of Tween 80.[6] The organisms were washed twice and resuspended in saline-Tween solution. The preparation was then centrifuged lightly to remove clumps of cells and the supernatant suspension was diluted in saline-Tween to equal 0.25 mg., wet weight, of the bacilli per ml. as determined with the Hopkins tube. The various other organisms used were grown on nutrient agar slopes for twenty-four hours at 37°C., collected in saline-Tween solution, and the suspensions were diluted to equal the wet weight value above. The bacterial inhibition test with *S. lutea* previously described (1) was used for the quantitative assay of lysozyme activity.

Stock solutions of crystalline egg-white lysozyme chloride were prepared in M/60 phosphate buffer, pH 6.8, sterilized by filtration through a Swinney filter, and stored at 4°C.[7]

[6] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

[7] The lysozyme used was the chloride form of crystalline egg-white lysozyme generously supplied by Armour and Company, Chicago, Illinois.

Titrations of the antibacterial activity of the various preparations tested were performed in 20 ml. screw-cap tubes containing Dubos broth (13), pH 7.0, plus 0.25 per cent of crystalline bovine albumin. One-tenth of a milliliter of the suspensions of organisms was used as the routine inoculum. In specified instances, either 0.25 per cent of bovine albumin fraction V or animal serum was used to replace the crystalline bovine albumin.[8] Incubation was carried out at 37°C. for one to two weeks with the tubercle bacilli and for one to four days with the other organisms tested. The inhibiting titer is expressed as the minimum amount of test substance per milliliter necessary to effect complete inhibition as judged by the absence of visible bacterial growth.

RESULTS

Effect of Serum on the Tuberculostatic Activity of Lysozyme

Early in the course of the work it was noted that the tuberculostatic activity of lysozyme for BCG was reduced when 4 per cent or more of bovine serum was added to the medium. This "serum effect" was not observed with *Mycobacterium phlei*.

The results of experiments on the serum effect, using various concentrations of bovine serum and crystalline bovine albumin at pH 7.0, are shown in table 1. Five per cent bovine serum produced the maximum serum effect, 3 per cent showed a slight effect, and 1 per cent was without effect. The serum effect with BCG was not observed when the serum was replaced with 0.25 per cent of crystalline bovine albumin. Since this concentration of albumin is greater than that contributed by the serum used, it appeared that some constituent in bovine serum other than albumin was responsible for the serum effect. The data in table 1 show that the serum effect with 5 per cent bovine serum was lost when the pH of the medium was raised from 7.0 to 7.5 This apparent influence of pH on the serum effect is the subject of further investigation.

It was first considered that the serum effect might be due to some constituent of serum which actually binds or destroys lysozyme. Accordingly, quantitative assays of lysozyme activity were conducted on the supernatants prepared from cultures of BCG grown for ten days in Dubos medium, pH 7.0, containing 5 per cent bovine serum and varying subinhibitive concentrations of lysozyme. These assays showed that 90 per cent or more of the original lysozyme activity of the media was found in the culture supernatants. Although this would appear to indicate that the serum effect does not result from the binding or destruction of lysozyme by the serum during the cultivation of the organisms, it is possible that binding by some component of the added serum actually took place but was largely reversed when the material was diluted for quantitative assay for lysozyme. Lysozyme may behave in a fashion similar to that observed in the serum and plasma protein binding of penicillin (14) and other drugs (15) in which the degree of binding is altered by dilution.

Another possibility considered was that the organisms adsorb some constituent from the serum which serves to protect them against lysozyme action. In order to determine whether or not the factor in serum responsible for the serum effect

[8] Crystalline bovine albumin and bovine albumin fraction V were purchased from Armour and Company, Chicago, Illinois.

could be removed by adsorption with cells, a sample of bovine serum was adjusted to pH 7.0 and adsorbed three times with one-tenth of its volume of washed cells of the BCG strain. The cells used for adsorption were washed twice in physiologic saline containing 0.04 per cent Tween 80. Tests of the ability of the "adsorbed" serum to produce the serum effect with BCG demonstrated that it was equally as effective as unadsorbed serum. The results are included in table 1.

In considering the matter further, it seemed probable that any substance in the serum which might be adsorbed to the cells and might protect them by blocking the union of lysozyme with its substrate would be of a basic character similar

TABLE 1

STUDIES ON THE SUPPRESSIVE ACTION OF SERUM ON THE TUBERCULOSTATIC ACTIVITY OF LYSOZYME

MICRO-ORGANISM	DUBOS MEDIUM PLUS	FINAL pH	BACTERIAL GROWTH				
			Lysozyme, γ per ml.				
			0	5	16	40	100
M. phlei	5 per cent bovine serum	7.5	4+	−	−	−	−
	5 per cent bovine serum	7.0	4+	−	−	−	−
	0.25 per cent crystalline bovine albumin	7.0	4+	−	−	−	−
BCG	5 per cent bovine serum	7.5	4+	±	−	−	−
	5 per cent bovine serum	7.0	4+	4+	4+	4+	3+
	3 per cent bovine serum	7.0	4+	4+	4+	3+	3+
	1 per cent bovine serum	7.0	4+	−	−	−	−
	5 per cent bovine serum (adsorbed with bentonite)	7.0	4+	4+	4+	4+	3+
	5 per cent bovine serum (adsorbed with BCG cells)	7.0	4+	4+	4+	4+	3+
	0.25 per cent crystalline bovine albumin	7.0	4+	−	−	−	−
	5 per cent bovine serum (inoculum washed in saline-Tween)	7.0	3+	2+	0	±	±

4+ represents growth equal to that of controls without lysozyme; range 1+ to 4+.
− represents no growth.
0 represents no trial.

to that of lysozyme. To explore this possibility, a 5 per cent volume of the anionic adsorbent bentonite (16) was added to a sample of serum. After ten minutes at room temperature, the bentonite was removed by centrifugation. Tests with the supernatant showed that the capacity of the serum to produce the serum effect had not been lessened by bentonite adsorption. The results are included in table 1. The failure to alter the activity of serum by adsorption with washed tubercle bacilli or with bentonite is presumptive evidence that the serum effect is not due to any factor in serum of a basic character or readily adsorbable by the cells themselves. It is of course possible that the serum factor is present in such large quantities that the adsorption with cells was not adequate.

Still another possibility to account for the serum effect is that the serum fulfills

a nutritive requirement which in some way enables the tubercle bacillus to synthesize a surface substance which protects it against lysozyme action.

Results indicating that a surface component is initially present on the cells of the inoculum, which is somehow necessary for expression of the full protective effect of serum, are included in the data presented in table 1. They show that the serum effect is lessened by the use of inocula composed of cells washed twice in saline containing 0.04 per cent of Tween 80. Further evidence that tubercle bacilli possess a surface component which reacts with lysozyme has been gained from the observation that, when young unwashed BCG cells from Sauton's medium are treated with high concentrations of lysozyme, they agglutinate strongly and present a thick "slime layer" under the electron microscope which is not readily observed in untreated cells (17). The surface material responsible for agglutination with lysozyme can be removed by washing several times with saline-

TABLE 2

ANTIBACTERIAL SPECTRUMS OF PROTAMINE SULFATE AND LYSOZYME

TEST ORGANISM	INHIBITING TITER PROTAMINE SULFATE γ PER ML.	INHIBITING TITER EGG-WHITE LYSOZYME γ PER ML.
BCG	500	4–5
Mycobacterium smegmatis	100	resistant*
M. phlei	50	4–5
S. lutea	50	0.2
B. subtilis	50	resistant*
Escherichia coli	50	resistant*

* Not inhibited by 200 to 500 γ per ml. of lysozyme. In the case of *B. subtilis* and *M. smegmatis*, 500 γ per ml. of lysozyme produced partial initial suppression of growth. However, it did not represent a sharp end-point titer, and further incubation resulted in near normal growth. Protamine, in contrast, produced a sharp end point of inhibition with these organisms and permanent suppression of growth.

Tween. The possible influence of the addition of serum to media upon the quantity or composition of the surface material has not been determined.

Experiments conducted to determine whether the property of bovine serum responsible for the serum effect is possessed by the serum of other animal species showed that the serum of the chicken, horse, rabbit, and human are all equally active.

Antibacterial Spectrums of Protamine Sulfate and Lysozyme

In order to explore the possibility that lysozyme may act by virtue of its basic character, the antibacterial spectrums of protamine sulfate and lysozyme were determined.[9] The results of the experiments are presented in table 2. They show that protamine sulfate differs markedly from lysozyme in its antibacterial spectrum. It is notable that the minimum inhibiting concentrations of protamine for

[9] The protamine clupeine sulfate was purchased from Nutritional Biochemicals Corp., 21010 Miles Avenue, Cleveland, Ohio.

most of the organisms tested were much greater than those for lysozyme. To illustrate, ten times as much protamine was required to inhibit BCG as to inhibit *M. phlei*, whereas both of these organisms were inhibited by the same quantity of lysozyme. Another observation presented in table 2 which suggests that the mechanism of action of the two substances is different is the contrast between the sharp end point of inhibition against *B. subtilis* and *M. smegmatis* produced by protamine and the partial inhibition observed with lysozyme.

Comparative Effect of Iodine on the Antibacterial Activity of Lysozyme and Protamine

As Meyer and associates (18) and Fraenkel-Conrat (19) reported that iodine in very small amounts can reversibly destroy the enzymatic activity of lysozyme, it appeared advisable to compare the effect of iodine on the antibacterial activity of lysozyme and protamine sulfate. Since the inactivation of lysozyme by iodine is reversible, the molecular alterations which accompany inactivation are probably minor. This, together with the fact that iodine apparently does not react with the epsilon amino groups of lysozyme (19), made it reasonable to expect that mild iodine treatment would not significantly alter the tuberculo-inhibitory activity of lysozyme were it due to basic character.

To test this hypothesis, 1,000 γ of lysozyme in 4.5 ml. of M/60 phosphate buffer, pH 6.8, were placed in each of four screw-cap tubes. To the first three tubes were added, respectively, 0.05, 0.1, and 0.2 ml. of a solution containing 0.3 per cent of iodine and 0.6 per cent of potassium iodide. The fourth tube served as a control. The final volume in each tube was adjusted to 5.0 ml. with M/60 phosphate buffer, pH 6.8. The tubes were allowed to stand at room temperature for one hour, and the solutions were assayed for lysozyme activity against *S. lutea* and *M. phlei*. The assays were conducted in small screw-cap vials, using 0.5 ml. volumes of various dilutions of each of the iodine-treated lysozyme solutions. One-half of a milliliter of melted nutrient agar (1 per cent agar), seeded with 0.005 mg. of the desired test organisms, was added to each diluted sample.

The results of the experiment are presented in table 3. They show that there is a direct relationship between the loss of bacteriostatic activity against *S. lutea* and *M. phlei* and the concentration of iodine used for treating the lysozyme solution. Iodine, even in low concentrations, was equally effective in suppressing the antibacterial activity of lysozyme against *S. lutea* and *M. phlei*. Controls containing only iodine in the final concentrations present in the lysozyme series showed that iodine alone had no influence on the growth of the test organisms used.

As the mode of action of lysozyme against *S. lutea* is enzymatic (2), the results provide suggestive evidence that the mode of action of lysozyme against *M. phlei* is likewise enzymatic.

In order to determine whether or not iodine can similarly suppress or destroy the antibacterial activity of protamine sulfate, an experiment was conducted in which an iodine solution similar to that above was added to each of a series of tubes containing 1 ml. of a 1 per cent solution of protamine sulfate. The volumes of the iodine solution added were 0.1, 0.2, 0.4, and 0.8 ml., respectively. Addi-

tional control tubes lacking iodine and protamine were included. The final volume in each tube was brought to 2.0 ml. by the addition of M/60 phosphate buffer, pH 6.8. After standing at room temperature for one hour, 0.5 ml. aliquots were removed from each tube and assayed for antibacterial activity against *M. phlei* and *S. lutea* by the procedure used above.

The results showed that the antibacterial activity of the protamine for the two organisms had not been changed by the iodine treatment. Titrations of samples of material from the control tubes in which iodine was included, but the protamine omitted, showed that the concentrations of iodine used did not affect the growth of the assay microorganisms. These results support the foregoing data

TABLE 3

THE EFFECT OF IODINE TREATMENT OF LYSOZYME ON ITS ANTIBACTERIAL ACTIVITY FOR S. LUTEA AND M. PHLEI

ML. OF IODINE SOLUTION USED FOR TREATMENT	ORIGINAL LYSOZYME CONCENTRATION			
	100	10	1	0
S. lutea				
0.05	−	−	+	+
0.1	+	+	+	+
0.2	+	+	+	+
Lysozyme control—no iodine treatment	−	−	−	+
M. phlei				
0.05	+	+	+	+
0.1	+	+	+	+
0.2	+	+	+	+
Lysozyme control—no iodine treatment	−	−	+	+

− = no growth.
+ = growth.

which indicate that the mechanism of the antibacterial action of protamine is different from that of lysozyme.

Cross-Resistance Tests with Strains of Organisms with Cultivated Resistance to Protamine and to Lysozyme

In an attempt to obtain further evidence on the modes of antibacterial action of protamine and lysozyme, strains of organisms resistant to the two respective substances were cultivated and cross-resistance tests were conducted.

Strains of *S. lutea* resistant to lysozyme, strains resistant to protamine, and strains with combined resistance to both lysozyme and protamine were developed by serial subculture in half-strength Dubos broth containing subminimal inhibiting concentrations of each of the two respective substances. The broth was diluted with an equal volume of physiologic saline to avoid the formation of a precipitate

which results when high concentrations of protamine are added to full-strength broth. In the case of the strain with combined resistance, the resistance to lysozyme was established before that to protamine.

Cross-resistance tests in which resistance to both protamine and lysozyme was determined on the parent and the three derived strains showed that the developed resistance was strictly specific. The minimum inhibiting level of lysozyme in gamma per milliliter proved to be 0.2 for the parent and the protamine-resistant strains, 2.0 for the lysozyme-resistant strain, and 2.0 for the lysozyme-protamine–resistant strain. It is especially notable that the development of protamine resistance in the lysozyme-resistant strain did not alter its lysozyme resistance. The minimum inhibiting level of protamine in gamma per milliliter was 50 for the parent and the lysozyme-resistant strains, 350 for the protamine-resistant strain, and 500 for the lysozyme-protamine–resistant strain.

Attempts to Develop Lysozyme Resistance in Mycobacteria

A limited number of trials were made to develop lysozyme-resistant strains of *M. phlei* and the Ravenel strain of *M. tuberculosis* var. *bovis* by treatment with tuberculocidal concentrations of lysozyme.

Heavy suspensions of the organisms containing 1.25 mg., wet weight, of bacilli per ml. were prepared for lysozyme treatment by washing twice in 0.04 per cent Tween-saline solution and suspending in Proskauer and Beck (P-B) broth, pH 7.0 (20). Lysozyme was added to give a final concentration of 5,000 γ per ml., and incubation was continued for ninety-six hours at 37°C. The suspensions were then cultured by dallying on plates of 1 per cent glycerol agar plus 5 per cent bovine serum.

With the Ravenel strain, the limited number of colonies which developed were more raised than the colonies of the parent strain and, upon first subculture in Dubos broth, the organisms grew in heavier clumps than the parent culture (17). This suggests that a surface change has occurred as the result of lysozyme treatment. When a number of the colonies were tested for their resistance to the tuberculostatic effects of lysozyme, none showed any increase in resistance over that of the parent strain (17). Thus, it would appear that whatever protective property was possessed by the organisms which survived the initial lysozyme treatment was ineffective in protecting the subsequent progeny against the tuberculostatic effects of lysozyme.

Inasmuch as the above treatment was conducted with lethal levels of lysozyme (3), the results do not exclude the possibility that resistant strains could be developed by repeated subculturing in subminimal bacteriostatic levels of lysozyme.

DISCUSSION

The observation that the addition of large amounts of serum to Dubos broth reduces the tuberculostatic activity of lysozyme for BCG was studied.

The mechanism of this "serum effect" remains unsettled. There is some indirect evidence that the protection demonstrated is due to a synthesis by the tubercle

bacillus of a surface substance which prevents lysozyme from reaching or acting upon its substrate in the cell wall. The results indicate that serum does not possess the capacity to destroy lysozyme or contain any substance which will adsorb to cells and protect them against lysozyme action. Although no positive evidence of serum binding of lysozyme was obtained, this possibility was not excluded.

It seems unlikely that the substrate is lacking in the cells grown in serum since it has even been found in species of microorganisms totally resistant to lysozyme (2). Lysozyme substrate may be a constituent of most, if not all, bacterial cells (2, 21). Indeed, it appears that the susceptibility of an organism to lysozyme may depend more upon the quantity and accessibility of the substrate in the cell wall than upon its presence or absence.

The observation that the pH of the medium may influence the serum effect was not studied sufficiently to permit any conclusions.

The marked difference between the antibacterial spectrums of protamine and lysozyme suggests that the mode of action of lysozyme is different from that of protamine. This is strengthened by the results of studies of strains of *S. lutea* with cultivated resistance to protamine and to lysozyme in which cross-resistance tests showed that the resistance of the respective cultivated strains of *S. lutea* is specific. Moreover, the observation that iodine destroys the antibacterial activity of lysozyme for *M. phlei* and *S. lutea* but does not destroy the activity of protamine indicates that the mode of action of lysozyme is distinct from that of protamine and suggests that it is solely enzymatic.

Despite the fact that the theories on the mechanism of the inactivation of lysozyme by iodine are conflicting, it does not appear that the inactivation is concerned with any alteration in the basic properties of lysozyme. Meyer and coworkers (18) believe that sulfhydryl groups of the lysozyme molecule must be intact for lysozyme activity and that iodine acts on lysozyme by oxidizing these groups. Fraenkel-Conrat (19), on the other hand, has presented evidence that iodine oxidizes the imino N of the imidiazole groups considered necessary for enzyme activity, as well as reacts with other groups such as phenolic groups to form iodo derivatives. It does not seem possible at present to evaluate these reports accurately since the critical factor of pH was not the same in all experiments. For example, Fraenkel-Conrat (19) performed most of his studies at pH 7.6, which favors the formation of iodo derivatives.

Other evidence which indicates that lysozyme does not exert tuberculocidal action through its basic radicals is that protamine, which is thought to act in this manner, does not produce any of the cytologic changes seen in tubercle bacilli treated with lysozyme (17). In view of the foregoing data, it seems highly probable that the antimycobacterial action of lysozyme does not depend in any measure upon a mechanism analogous to that of protamine but, instead, is solely enzymatic.

SUMMARY

Tubercle bacilli were observed to be resistant to the tuberculostatic effects of lysozyme when grown in Dubos broth plus 5 per cent serum at pH 7.0. The factor responsible for this "serum effect" was not adsorbed from the serum by treatment

with bentonite or with washed cells of the BCG strain. The serum effect was not observed with *M. phlei*.

Iodine was observed to inactivate the antibacterial property of lysozyme for *M. phlei* as well as for *S. lutea*, whereas it did not alter the antibacterial property of protamine for these organisms.

Marked differences were noted between the antibacterial spectrums of lysozyme and protamine.

Cross-resistance tests conducted on strains of *S. lutea*, with cultivated resistance to lysozyme and to protamine, showed that resistance of the organisms to the respective substances is specific.

Attempts to obtain strains of *Mycobacteria* with increased resistance to the bacteriostatic effects of lysozyme by treatment with tuberculocidal levels of lysozyme were unsuccessful.

The foregoing data indicate that the action of lysozyme on tubercle bacilli is not of a nonspecific character attributable, as in the case of protamine, to basic properties but instead is that of an enzyme.

SUMARIO

Estudios del Modo de Acción de la Lisozima sobre las Micobacterias

Según se observó, los bacilos tuberculosos eran resistentes a los efectos tuberculostáticos de la lisozima al ser cultivados en caldo de Dubos más 5 por ciento de suero a un pH de 7.0. El factor causante de dicho "efecto sérico" no fué adsorbido del suero por tratamiento con bentonita o con células lavadas de la cepa BCG. El efecto sérico no fué observado en el *M. phlei*.

Observóse que el yodo inactivaba la propiedad antibacteriana de la lisozima para el *M. phlei* así como para la *S. lutea*, en tanto que no alteraba la propiedad antibacteriana de la protamina para dichos microbios.

Notáronse diferencias pronunciadas entre las propiedades antibacterianas de la lisozima y la protamina.

Una prueba de la resistencia cruzada ejecutada en cepas de *S. lutea*, con resistencia cultivada a la lisozima y la protamina, demostró que es específica la resistencia de los gérmenes a las respectivas substancias.

Resultaron infructuosos los esfuerzos encaminados a obtener cepas de micobacterias dotadas de mayor resistencia a los efectos bacteriostáticos de la lisozima por tratamiento con dosis tuberculocidas de lisozima.

Los datos anteriores indican que la acción de la lisozima sobre los bacilos tuberculosos no es de naturaleza anespecífica imputable, como sucede con la protamina, a propiedades básicas, sino que es la de una enzima.

RESUME

Etudes sur le mode d'action de la lysozyme sur les mycobactéries

Les bacilles tuberculeux se sont montrés résistants à l'action tuberculostatique de la lysozyme quand ils étaient cultivés sur un milieu Dubos avec 5% de sérum au pH7. Le facteur responsable de cet "effet du sérum" n'était pas absorbé à partir du sérum lorsqu'on pratiquait le traitement par la bentonite ou par les cellules lavées de la souche DCG. L'"effet du sérum" n'était pas observé avec le *M. phlei*.

Les auteurs ont observé que l'iode inactivait la propriété antibactérienne de la lysozyme sur le *M. phlei* et sur le *S. lutéa* alors qu'elle n'altérait pas la propriété antibactérienne de la protamine pour ces microorganismes.

Des différences marquées ont été observées entre les spectres d'action antibactérienne de la lysozyme et de la protamine. Le test de résistance croisée effectué sur des souches de

S. lutéa, chez lesquelles une résistance à la lysozyme et à la protamine avait été introduite, a démontré que la résistance des organismes à ces substances respectives est spécifique.

Des tentatives effectuées pour obtenir des souches de mycobactéries possédant une augmentation de la résistance aux effets bactériostatiques de la lysozyme par un traitement avec des concentrations tuberculocides de lysozyme ont échoué.

Les données précédentes indiquent que l'action de la lysozyme sur le bacilles tuberculeux est d'ordre enzymatique et non une reaction specifique attribuable a des propriétés basiques comme c'est le cas pour la protamine.

REFERENCES

(1) Myrvik, Q., and Weiser, R. S.: A tuberculostatic serum substance possessing lysozyme-like properties, Am. Rev. Tuberc., 1951, *64*, 669.

(2) Epstein, L. A., and Chain, E.: Some observations on the preparation and properties of the substrate of lysozyme, Brit. J. Exper. Path., 1940, *21*, 339.

(3) Myrvik, Q., Weiser, R. S., and Agar, H.: Lethal and cytologic effects of lysozyme on tubercle bacilli, Am. Rev. Tuberc., 1953, *67*, 217.

(4) McClean, D.: Further observations on testicular extract and its effect upon tissue permeability, J. Path. & Bact., 1931, *34*, 459.

(5) Miller, B. F., Abrams, R., Dorfam, A., and Klein, M.: Antibacterial properties of protamine and histone, Science, 1942, *96*, 428.

(6) Negroni, P., and Fischer, I.: Sobre la accion antibiotica de las protaminas e histonas, Rev. Soc. argent. de biol., 1944, *20*, 307.

(7) Bloom, W. L., Winters, M. G., and Watson, D. W.: The inhibition of two antibacterial basic proteins by nucleic acids, J. Bact., 1951, *62*, 7.

(8) Steudel, H., and Peiser, E.: Uber Nucleinsaure: Eiwei verbindungen, Ztschr. f. physiol. Chem., 1922, *122*, 208.

(9) Neuberg, C., and Roberts, I. S.: Remarkable properties of nucleic acids and nucleotides, Arch. Biochem., 1949, *20*, 185.

(10) Rubini, J. R., Rasmussen, A. F., and Stahmann, M. A.: Inhibitory effect of synthetic lysine polypeptides on growth of influenza virus in embryonated eggs, Proc. Soc. Exper. Biol. & Med., 1951, *76*, 662.

(11) Fletcher, W., Gulland, J. M., and Jordan, D. O.: The constitution of yeast ribonucleic acid: VIII. Electrometric titration of acid groups, J. Chem. Soc. London, 1944, *147*, 33.

(12) Gulland, J. M., and Jordan, D. O.: Structure of yeast ribonucleic acid, Nature, 1948, *161*, 561.

(13) Dubos, R. J., and Middlebrook, G.: Media for tubercle bacilli, Am. Rev. Tuberc., 1947, *56*, 334.

(14) Tompsett, R., Schultz, S., and McDermott, W.: The relation of protein binding to the pharmacology and antibacterial activity of penicillins X, G, dihydro F, and K, J. Bact., 1947, *53*, 581.

(15) Davis, B. D.: Physiological significance of the binding of molecules by plasma proteins, Am. Scientist, 1946, *34*, 611.

(16) Alderton, G., Ward, W. H., and Fevold, H. L.: Isolation of lysozyme from egg white, J. Biochem., 1945, *157*, 43.

(17) Myrvik, Q., and Weiser, R. S.: Unpublished work.

(18) Meyer, K., Thompson, R., Palmer, J. W., and Khorazo, D.: The purification and properties of lysozyme, J. Biol. Chem., 1936, *113*, 479.

(19) Fraenkel-Conrat, H.: The essential groups of lysozyme, with particular reference to its reaction with iodine, Arch. Biochem., 1950, *27*, 109.

(20) Proskauer, B., and Beck, M.: Beitrage fur Ernahrungsphysiologie des Tuberkelbacillus, Ztschr. f. Hyg. u. Infektionskr., 1894, *18*, 128.

(21) Webb, M.: The action of lysozyme on heat-killed gram-positive microorganisms, J. Gen. Microbiol., 1948, *2*, 261.

THE EFFECT OF COMBINED THERAPY WITH ISONIAZID AND STREPTOMYCIN ON EXPERIMENTAL TUBERCULOSIS OF GUINEA PIGS[1]

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INTRODUCTION

The occurrence of isoniazid-resistant tubercle bacilli in the early clinical trials of the drug prompted appropriate warnings that this new chemotherapeutic agent should be used in combination with other known antituberculous drugs (1–3). It was subsequently reported that "the emergence of bacterial resistance during daily isoniazid therapy has been delayed by the concomitant administration of intermittent streptomycin" (4). In addition to this desirable result of using isoniazid and streptomycin concomitantly, it is of importance to learn whether combined therapy with these drugs is therapeutically advantageous. It was recently reported (5) that, after twenty weeks of treatment, there was roentgenographic evidence of greater improvement among patients treated with streptomycin plus isoniazid than among those given streptomycin plus *p*-aminosalicylic acid. There was only little evidence of any superiority of streptomycin plus isoniazid over the use of isoniazid alone.[2, 3]

The experiment reported herein was designed to compare the therapeutic effect on experimental tuberculosis in guinea pigs of isoniazid alone and in combination with streptomycin, using doses of each drug which, individually, were known to effect incomplete but measurable regression of the disease. It was found that the combined use of isoniazid and streptomycin was therapeutically superior to the use of either drug alone in comparable doses.

METHODS

Sixty-eight mature, male guinea pigs, weighing 700 to 800 gm. each, were each inoculated subcutaneously over the sternum with 0.1 mg. (moist weight) of virulent human-type tubercle bacilli (H37Rv). On the twenty-second day, 6 were killed and were found to have grossly visible lesions of tuberculosis at the site of inoculation, axillary lymph nodes, spleen,

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[1] From the Section of Pathologic Anatomy, Mayo Foundation and Mayo Clinic, Rochester, Minnesota.

[2] After completion of this paper, there appeared a report of the British Medical Research Council (Brit. M. J., 1953, No. 4809, 521) comparing the clinical results of treatment with streptomycin plus isoniazid, streptomycin plus *p*-aminosalicylic acid, and isoniazid alone in a series of 364 patients. It was concluded, "judging solely from the results at three months, that streptomycin plus isoniazid with the doses used is clinically the most effective of the treatments studied . . . ."

[3] Since completion of this paper, Mount, Jenkins, and Ferebee (Am. Rev. Tuberc., 1953, *68*, 264) have concluded that "Among 583 patients with streptomycin-susceptible infections treated for forty weeks with streptomycin-PAS, streptomycin-isoniazid, or isoniazid alone, those receiving streptomycin-isoniazid maintained a small but consistent advantage on the basis of roentgenographic, clinical, and laboratory findings.

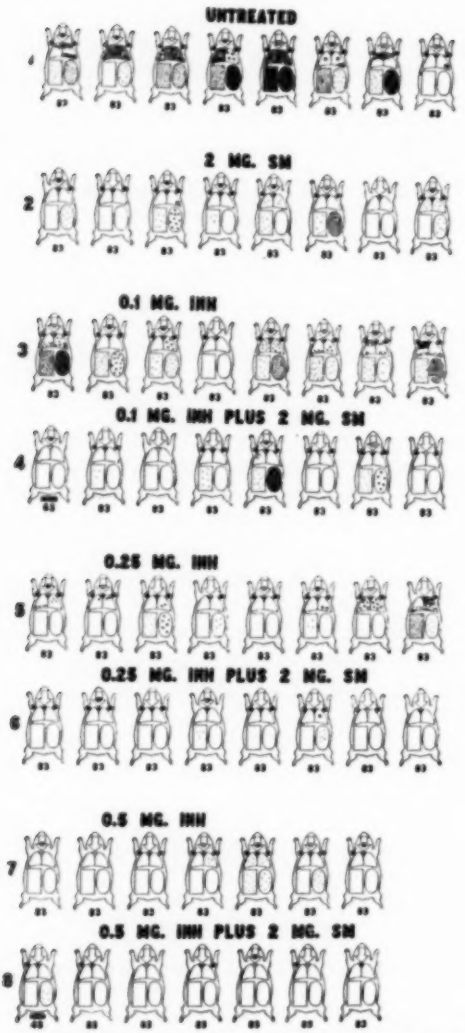

FIG. 1. Schematic representation of the tuberculous lesions seen in each animal at necropsy. The rectangle and oval represent liver and spleen, respectively. Miliary and nodular lesions are represented by dots. Blackening of an organ indicates diffuse tuberculous involvement. A black bar indicates that the animal died. All other animals were killed on the eighty-third day of infection. Treatment started on the twenty-second day of infection and continued for sixty-one days. The amounts of drug shown were given once daily. SM = streptomycin, INH = isoniazid.

liver, and tracheobronchial lymph nodes. Three had minute lesions in the lungs. The remaining 62 animals were divided into eight groups as shown in figure 1.

On the twenty-second day of infection, groups of treated animals were started on a course of treatment with streptomycin alone and with isoniazid[4] alone or in combination as shown in figure 1. The amounts shown were given once daily in 1 ml. of aqueous solution. The streptomycin was given by injection into the axillary space. The isoniazid was given by stomach tube and was administered within five minutes after injection of streptomycin.

The daily dose of 2 mg. of streptomycin was selected because this amount of streptomycin administered daily for approximately sixty days will cause only partial but measurable regression and healing of tuberculous lesions in guinea pigs (6). Likewise, the doses of isoniazid, 0.1 mg., 0.25 mg., and 0.5 mg., respectively, were used because these amounts of the drug are only partially effective as compared with doses of 1.0 mg. or more when given daily for a period of sixty days (7).

After sixty-one days of treatment, all animals, including the untreated controls, were killed. A schematic record was made of the macroscopically evident lesions of tuberculosis as shown in figure 1. Portions of lungs, liver, kidneys, and spleen and the site of inoculation and axillary nodes were preserved for subsequent histopathologic examination. A portion of spleen was selected for culture if this organ had grossly visible lesions. A visibly enlarged axillary lymph node was selected for culture when the spleen appeared to be normal. All positive cultures were subsequently tested for resistance to streptomycin and to isoniazid.

RESULTS

Necropsy observations: It may be seen in figure 1 that all of the untreated animals (*Group 1*), with the exception of one animal, had extensive lesions of tuberculosis. In contrast, those given 2 mg. of streptomycin daily (*Group 2*) had much less visible disease, but only 2 of the 8 animals had no evidence of lesions in the lungs, liver, or spleen. The effect of administering 0.1 mg. of isoniazid was not impressive with respect to the arrest of the disease process (*Group 3*). The results achieved by administering 0.1 mg. of isoniazid plus 2 mg. of streptomycin (*Group 4*) suggest, perhaps, that there may have been some increased therapeutic effect. However, the results found at necropsy for *Group 4* may well be ascribed to the effect of the 2 mg. of streptomycin alone.

In *Group 5*, in which the animals were given 0.25 mg. of isoniazid once daily, one animal had no grossly visible lesions in the lungs, liver, or spleen and, with one exception, the others had definitely less tuberculosis than the controls. The therapeutic effect appears to be comparable to that obtained by administering 2 mg. of streptomycin (*Group 2*) alone, in which 2 animals had no lesions in the internal organs.

The administration of 0.25 mg. of isoniazid plus 2 mg. of streptomycin (*Group 6*) produced results which were unequivocally better than those produced by either drug alone at these doses. Six animals in this group had no visible lesions in the lungs, liver, or spleen, and 2 had minimal involvement. This result resembled those previously reported from this laboratory which were achieved by administering 1.0 mg. or more of isoniazid daily (7) or 6 mg. or more of streptomycin daily (6).

The administration of 0.5 mg. of isoniazid alone (*Group 7*) effected such marked regression of the disease, as determined grossly, that it was difficult to assess the value of using 0.5 mg. of isoniazid plus 2 mg. of streptomycin (*Group 8*). Although only one animal in the latter group had visible lesions in an internal

[4] The isoniazid was furnished by E. R. Squibb & Sons, New Brunswick, New Jersey.

organ as compared with 3 in *Group 7*, the differences between the two groups are not impressive.

Microscopic examination: Histopathologic studies of the lungs, liver, spleen, and kidneys disclosed no changes attributable to any toxic effects of the treatment. With respect to the tuberculous lesions, the microscopic appearance confirmed the observations made at necropsy. However, there appeared to be some differences between groups which were not discernible grossly. This was found by determining the percentage of the total number of tissues in each group which had active lesions, inactive lesions, or no demonstrable lesions as shown in table 1.

TABLE 1

PERCENTAGE OF THE TOTAL NUMBER OF TISSUES FROM THE ANIMALS IN EACH GROUP WHICH HAD MICROSCOPIC EVIDENCE OF ACTIVE LESIONS, INACTIVE LESIONS, OR NO LESIONS*

GROUP	DAILY TREATMENT	NUMBER OF ANIMALS	TOTAL NUMBER OF TISSUES†	RESULTS OF MICROSCOPIC EXAMINATION		
				Per Cent Active‡	Per Cent Inactive§	Per Cent with No Lesions
1	None	8	32	97	3	None
2	2 mg. of SM§‖	8	32	63	12	25
3	0.1 mg. of INH‖	8	32	88	12	None
4	2 mg. of SM plus 0.1 mg. of INH	8	32	44	16	40
5	0.25 mg. of INH	8	32	66	12	22
6	2 mg. of SM plus 0.25 mg. of INH	8	32	38	22	40
7	0.5 mg. of INH	7	28	32	21	47
8	2 mg. of SM plus 0.5 mg. of INH	7	28	18	32	50

* The four common sites of predilection, that is, lungs, liver, spleen, and the site of inoculation (including axillary lymph nodes), were examined. In addition to large destructive lesions, small lesions with beginning fibrosis but with residual necrotic foci were also classed as active. The latter were encountered particularly in the axillary lymph nodes of treated animals.

† Lungs, liver, spleen, and site of inoculation from each animal.

‡ Active lesions = necrotic foci.

§ Inactive lesions = fibrosis or calcification or epithelioid cell tubercles with no necrosis.

‖ SM = streptomycin; INH = isoniazid.

It may be seen that, for each group which was given isoniazid plus 2 mg. of streptomycin (*Groups 4*, *6*, and *8*), there were fewer tissues with active necrotic lesions as compared with the group given 2 mg. of streptomycin alone (*Group 2*) and the corresponding groups given isoniazid alone (*Groups 3*, *5*, and *7*). In many of the treated animals in *Groups 6*, *7*, and *8*, the actively progressive and necrotic lesions were confined to residual foci in the axillary lymph nodes.

An index of infection was determined microscopically for each group. This was based on the extent and character of the tuberculous lesions in the lungs, liver, spleen, and site of inoculation. In this method of evaluation, values of 10 to 35, depending on the extent of involvement, are given to organs with active necrotic lesions and values of 1 to 3 are given when only fibrosis, calcification, or

epithelioid cell tubercles with no necrosis are present. The average for each group is presented in figure 2, which shows graphically that there was less microscopic evidence of active tuberculous disease in each of the groups which were given the combined treatment than when the drugs were used alone in comparable doses.

Microbiologic studies: Cultures obtained from the spleen or an axillary lymph node of the treated animals in all groups as well as from the untreated animals were susceptible *in vitro* to more than 1.0 γ of streptomycin and to more than 0.04 γ of isoniazid per ml. of egg-yolk–agar medium. The failure to find isoniazid-

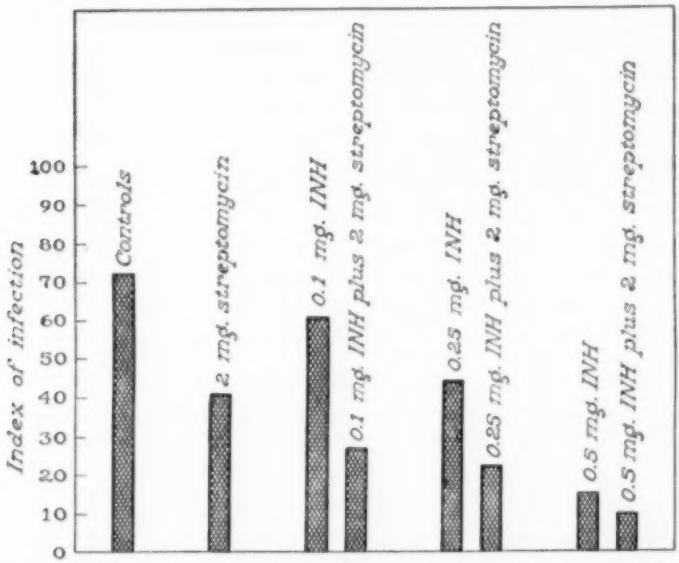

FIG. 2. Average index of infection. Values of 10 to 35 are given to organs with active tuberculous lesions, depending on the extent of involvement. Values of 1 to 3 are given when the lesions are inactive as indicated by fibrosis, calcification, and the absence of necrosis. Treatment started twenty-two days after infection and continued for sixty-one days. The amounts of drug shown were given once daily. INH = isoniazid.

resistant tubercle bacilli in guinea pigs which were treated for approximately sixty days with the drug is in agreement with the previous experience (7).

COMMENT

In experiments such as the present one with antimicrobial drugs of relatively great activity, it is necessary that the dose of each drug used in combined therapy be adjusted so that one of the drugs alone cannot be expected to produce its maximal therapeutic effect (8). For example, in *Group 7*, the administration of 0.5 mg. of isoniazid resulted in sufficient regression and healing of the disease so that there was only slightly increased effect of giving 2 mg. of streptomycin.

Had the studies been limited to regimens employing 0.5 mg. or more of isoniazid, the results of the combined treatment would have been difficult to assess. However, at doses of 0.25 mg. and even 0.1 mg. of isoniazid plus 2 mg. of streptomycin, there is ample evidence that the combined use of these two drugs had a greater beneficial effect than did either drug alone.

Of interest was the observation that the microscopic study disclosed certain differences between the groups which were not readily apparent by necropsy examination alone. This may be due to finding microscopically small active foci not grossly visible and also to the inability to distinguish grossly between actively progressive lesions and those with beginning fibrosis and healing.

The failure to recover isoniazid-resistant tubercle bacilli from guinea pigs which were treated with the drug is similar to the experience with streptomycin in the treatment of experimental tuberculosis of guinea pigs. Steenken (9) has reported that in his experiments no streptomycin-resistant form of tubercle bacilli was found in guinea pigs which were treated for 125 days. Likewise, streptomycin-resistant tubercle bacilli were not encountered in the guinea pigs of the present study except in 3 of 8 animals which were treated with streptomycin for 144 days and in 5 of 6 guinea pigs treated for 215 days (10). Perhaps isoniazid-resistant tubercle bacilli may occur in tuberculous guinea pigs after prolonged therapy. Only under such conditions would it be possible to test in guinea pigs the effect of combined treatment with streptomycin on the emergence of streptomycin- or isoniazid-resistant strains.

SUMMARY

Twenty-two days after being infected with virulent tubercle bacilli, eight groups of guinea pigs were started on daily treatment for sixty-one days as follows:

Group 1, controls (untreated); *Group 2*, 2 mg. of streptomycin; *Group 3*, 0.1 mg. of isoniazid; *Group 4*, 0.1 mg. of isoniazid plus 2 mg. of streptomycin; *Group 5*, 0.25 mg. of isoniazid; *Group 6*, 0.25 mg. of isoniazid plus 2 mg. of streptomycin; *Group 7*, 0.5 mg. of isoniazid; *Group 8*, 0.5 mg. of isoniazid plus 2 mg. of streptomycin.

The necropsy findings indicated that the administration of 0.1 mg. of isoniazid plus 2 mg. of streptomycin was only slightly more beneficial than 2 mg. of streptomycin alone. Microscopic examination, however, disclosed unequivocal evidence that, at these doses, the combined use of these drugs resulted in greater regression of the lesions than was produced by either drug alone. This desirable effect was most pronounced in the group treated with 0.25 mg. of isoniazid and 2 mg. of streptomycin. The administration of 0.5 mg. of isoniazid alone resulted in such marked diminution of the lesions that, when 0.5 mg. of isoniazid was used in combination with 2 mg. of streptomycin, there was only a slightly increased beneficial effect. Bacteriologic study failed to disclose isoniazid-resistant or streptomycin-resistant tubercle bacilli from any animal which was treated with either drug alone or in combination.

SUMARIO

El Efecto de la Terapéutica Combinada con Isoniacida y Estreptomicina sobre la Tuberculosis Experimental del Cobayo

A los veintidós días de infectar con bacilos tuberculosos virulentos a ocho grupos de cobayos, se les aplicó tratamiento diario durante sesenta y un días en la forma siguiente: *Grupo 1* (testigos), sin tratar; *Grupo 2*, 2 mg. de estreptomicina; *Grupo 3*, 0.1 mg. de isoniacida; *Grupo 4*, 0.1 mg. de isoniacida más 2 mg. de estreptomicina; *Grupo 5*, 0.25 mg. de isoniacida; *Grupo 6*, 0.25 mg. de isoniacida más 2 mg. de estreptomicina; *Grupo 7*, 0.5 mg. de isoniacida; *Grupo 8*, 0.5 mg. de isoniacida más 2 mg. de estreptomicina.

Los hallazgos autópsicos indicaron que la administración de 0.1 mg. de isoniacida más 2 mg. de estreptomicina fué apenas un poco más beneficiosa que 2 mg. de estreptomicina sola. Sin embargo, el examen microscópico reveló pruebas indudables de que, con dichas dosis, el empleo combinado de esas drogas logró mayor regresión de las lesiones que la producida por una u otra droga por sí sola. Ese efecto favorable fué más notable en el grupo tratado con 0.25 mg. de isoniacida y 2 mg. de estreptomicina. La administración de 0.5 mg. de isoniacida sola dió por resultado una disminución tan acentuada de las lesiones que, al usar 0.5 mg. de isoniacida en combinación con 2 mg. de estreptomicina, apenas aumentó ligeramente el efecto beneficioso. El estudio bacteriológico no reveló bacilos tuberculosos isoniacidorresistentes o estreptomicinorresistentes procedentes de ningún animal tratado con una u otra droga, ya solas o combinadas.

RESUME

L'effet de la thérapie associée Isoniazide-Streptomycine dans la tuberculose expérimentale du cobaye

Vingt-deux jours après inoculation avec des bacilles tuberculeux, huit groupes de cobayes ont été soumis pendant soixante-et-un jours à un traitement quotidien dont voici le détail: *Groupe 1* (témoins) non traité, *Groupe 2*: 2 mg. de streptomycine, *Groupe 3*: 0,1 mg. d'isoniazide, *Groupe 4*: 0,1 mg. isoniazide plus 2 mg. de streptomycine, *Groupe 5*: 0,25 mg. d'isoniazide, *Groupe 6*: 0,25 mg. d'isoniazide plus 2 mg. de streptomycine, *Groupe 7*: 0,5 mg. d'isoniazide, *Groupe 8*: 0,5 mg. d'isoniazide plus 2 mg. de streptomycine.

Les résultats d'autopsie montrent que l'efficacité de l'administration de 0,1 mg. d'isoniazide plus 2 mg. de streptomycine n'était guère plus marquée que celle de 2 mg. de streptomycine seule; l'examen microscopique a cependant fourni la preuve que l'association de ces produits aux doses utilisées déterminait une régression des lésions plus marquée que celle qui était observée avec l'une ou l'autre de ces substances administrée séparément.

C'est dans le groupe traité avec 0,25 mg. d'isoniazide et 2 mg. de streptomycine que cet effet a été le plus accentué. L'administration de 0,5 mg. d'isoniazide seule a provoqué une telle réduction des lésions que l'effet favorable n'a guère été accru lorsqu'à 0,5 mg. d'isoniazide on a associé 2 mg. de streptomycine. Les études bactériologiques n'ont pas révélé bacilles tuberculeux isoniazid-résistants ou streptomycino-résistants chez les animaux traités avec ces substances associées, ou administrées séparément.

REFERENCES

(1) Steenken, W., Jr., Meade, G. M., Wolinsky, E., and Coates, E. O.: Drug-resistant tubercle bacilli from patients treated with isonicotinic acid hydrazides, J.A.M.A., 1952, *149*, 187.

(2) Rist, N.: La resistance du bacille tuberculeux à l'hydrazide iso-nicotinique, Presse méd., 1952, *60*, 806.

(3) Grace, E. J., Bryson, V., Szybalski, W., and Demerec, M.: Potential danger of isoniazid resistance through failure to use multiple chemotherapy in treatment of tuberculosis, J.A.M.A., 1952, *149*, 1241.

(4) Dye, W. E., Lynch, H. P., and Brees, A. G.: Incidence of bacterial resistance encountered with tuberculosis chemotherapy regimens employing isoniazid alone and in combination with intermittent streptomycin, Am. Rev. Tubere., 1953, *67*, 106.

(5) Mount, F. W., and Ferebee, S. H.: Control study of comparative efficacy of isoniazid, streptomycin-isoniazid, and streptomycin–para-aminosalicylic acid in pulmonary tuberculosis therapy: II. Report on twenty-week observations on 390 patients with streptomycin-susceptible infections, Am. Rev. Tubere., 1953, *67*, 108.

(6) Karlson, A. G., and Feldman, W. H.: The subeffective dose of streptomycin in experimental tuberculosis of guinea pigs, Am. Rev. Tubere., 1948, *58*, 129.

(7) Karlson, A. G., and Feldman, W. H.: Effective therapeutic dose of isoniazid for experimental tuberculosis of guinea pigs, Am. Rev. Tubere., 1953, *68*, 75.

(8) Karlson, A. G., and Feldman, W. H.: The use of guinea pigs in studying chemotherapy of experimental tuberculosis, Ann. New York Acad. Sc., 1949, *52*, 637.

(9) Steenken, W., Jr.: Streptomycin in guinea pig tuberculosis, Am. Rev. Tubere., 1947, *56*, 382.

(10) Feldman, W. H., and Karlson, A. G.: Streptomycin in experimental tuberculosis, in Waksman, S. A.: Streptomycin; Nature and Practical Applications, The Williams and Wilkins Company, Baltimore, 1949, pp. 133–157.

USE OF THE GUINEA PIG OMENTUM AS AN INDEX OF THE EFFECTIVENESS OF ANTIMICROBIALS IN EXPERIMENTAL TUBERCULOSIS[1]

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INTRODUCTION

One of the difficulties which has to be faced in the experimental evaluation of tuberculostatic agents is the length of time required for the evolution of the tuberculous process. Thus, in the classic experiment of Feldman, Hinshaw, and Mann with streptomycin (1), the subcutaneously infected guinea pigs were allowed to live for 215 days before they were sacrificed. Likewise, in animals infected directly in the lungs, using air-borne bacilli (2, 3), the evolution of tuberculosis is slow. Steenken and Wolinsky (4) have found a more rapid development of the disease after the intracerebral infection of guinea pigs, most of the untreated control animals being dead after three weeks. Youmans and McCarter (5), using intravenously infected mice as experimental animals, were able to terminate the experiment after twenty-eight days. It is evident that any procedure which would make possible the evaluation of a new antimicrobial agent in a shorter period of time would be very useful, particularly in the initial screening of drugs.

Recently Suter (6, 7) and Mackaness and Smith (8–10) have shown that cell cultures infected with tubercle bacilli may be used for testing the effect of antimicrobials, the results of these tests being available after only a few days. This work with cell cultures suggested a previous study in which guinea pigs were inoculated intraperitoneally with tubercle bacilli and there was created for all practical purposes an infected cell culture in which tubercle bacilli grew for a time in complete symbiosis with the cells of the omentum (11). Thus, the peritoneal cavity might be regarded as a large *in vivo* culture tube with certain obvious advantages over more artificial types of cell cultures. One of these advantages is the fact that the inoculated tubercle bacilli are swept up in large part by the omentum, which, as the disease develops, becomes converted into a large sausage-shaped mass in approximately two weeks' time (12, 13).

Some ten years ago, Saz and his co-workers (14) pointed out that, in the evaluation of chemotherapeutic agents, the ratio of the weight of the excised omentum to the weight of the intraperitoneally infected guinea pig might be used to indicate the effectiveness of the agent which had been employed. The present writers have been unable to find any subsequent reference to the use of this method, possibly because of the rather complicated procedure suggested by Saz. Accordingly, the present study was conducted with the idea of exploring

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further the possibility of using the peritoneal cavity as a sort of natural test tube in antimicrobial tests and of using the ratio of the omental weight to the body weight as an index of the effectiveness of the therapeutic agent.

MATERIAL AND METHODS

Young albino guinea pigs, most of them 250 to 300 gm. in weight, were inoculated intraperitoneally with 0.1 mg. of virulent tubercle bacilli (H37Rv)[3] suspended in 0.5 ml. of sterile saline. In making intraperitoneal inoculations in the guinea pig, the hazard of penetrating

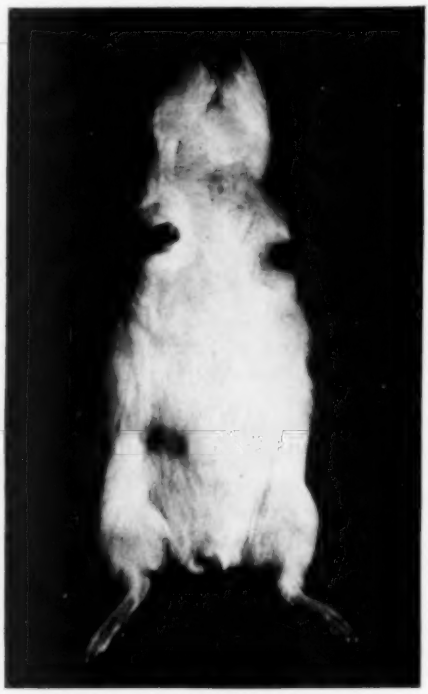

FIG. 1. Guinea pig sacrificed forty-eight hours after an intraperitoneal inoculation. The small skin incision has healed spontaneously.

the huge appendix or a loop of gut and thus losing the inoculum is always present (14, 15). This was guarded against by plucking the hair from a small area a few millimeters to the right of the midpoint of the abdomen. A fold of skin in this area was lifted and then cut across with sharp-pointed scissors, thus exposing the abdominal muscles. The muscles and parietal peritoneum were readily penetrated with a 24-gauge inoculating needle which was made slightly dull by rubbing the point on an abrasive. The skin incisions closed spontaneously (figure 1).

The inoculum was made from crumbs of tubercle bacilli removed from a fourteen-day-old

[3] Obtained from the National Tuberculosis Association Standard Culture Depot, Trudeau, New York.

culture on Löwenstein medium. The weighed bacilli were carefully ground with saline, using a mortar and pestle. No attempt was made to remove the relatively large clumps of bacilli remaining in the inoculum, since these are broken up by phagocytosis (11). In a suspension such as this, the bacilli sediment rapidly. To obtain uniform inoculation of the various guinea pigs, the inoculum for only one animal at a time was taken into the syringe, the suspension being mixed thoroughly before each filling of the syringe.

Chemotherapy was started within half an hour of the time the animals were inoculated and was continued with one dose each day. Streptomycin (calcium chloride complex) in saline solution was given at the rate of 40 mg. per kg. of body weight. The solution contained 10 mg. of streptomycin per ml. The animals which received isoniazid were divided into two groups, one group receiving the drug at the rate of 10 mg. per kg. In the other group the dose was 3 mg. per kg. Isoniazid in saline solution was used, the first solution containing 5 mg. per ml; the second, 1.5 mg. per ml. The chemotherapeutic agents were injected into the axillary region, the right and left axilla being used on alternate days.

FIG. 2. Omentum, pancreas, and spleen of the guinea pig. Organs on the left are from an animal which weighed 600 gm.; on the right, from one which weighed 210 gm.

Most of the animals were weighed and sacrificed on the fourteenth day, following which the omentum was removed and weighed.[4] Care was taken to exclude the pancreas which, in the guinea pig, fans out in the omentum from the region of the splenic hilum (figure 2). Presumably because of its natural immunity, the pancreas is not involved during the first few weeks after the intraperitoneal inoculation of tubercle bacilli. Its gray-pink color makes it readily distinguishable from the tuberculous omentum. In figure 2 also is illustrated one of the advantages of using small guinea pigs, namely, the smaller amount of perivascular fat which has accumulated in the omentum.

Omental index: The omental index was determined by dividing the weight of the omentum in grams by the weight of the guinea pig in kilograms. In the normal uninfected guinea pig,

[4] The autopsies should be performed under a safety hood. The omentum is dropped onto a square of *parafilm* in a Petri dish and (without the Petri dish) is weighed to the nearest 0.01 gm. on a chemical balance. The square of parafilm may be weighed separately, or squares of uniform size may be used and assumed to be of similar weight, within the limits of accuracy of the experiment.

this ratio varies from 0.39 (average of 6 pigs) in animals which weigh 200 to 300 gm. to 0.55 or more in pigs which weigh more than 500 gm. Thus, in a given experimental series, it is important to have animals of approximately the same size.[5]

RESULTS

Effect of streptomycin: Streptomycin (40 mg. per kg.) was first tried on 20 guinea pigs infected intraperitoneally in the manner described. At twenty-four hours and then on alternate days a treated pig and an infected control animal were sacrificed. Using a well-known technique (11, 13), spread preparations of the omentum were made on microscopic slides and stained by the Ziehl-Neelsen method. Examined under the microscope, these preparations revealed that most of the acid-fast bacilli had been phagocytosed by polymorphonuclear leukocytes

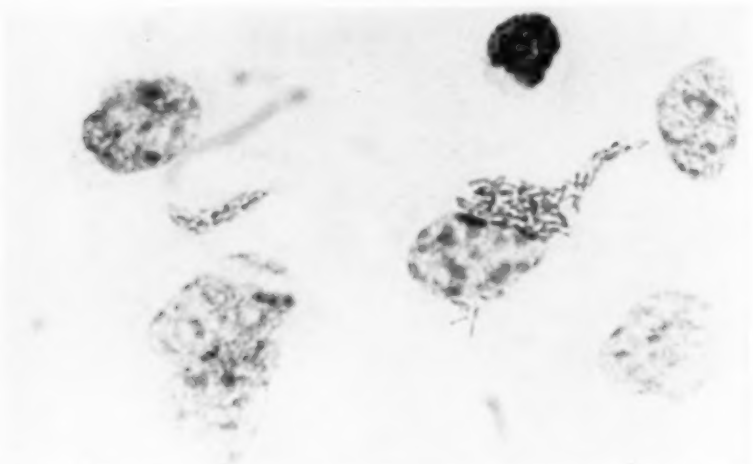

FIG. 3. Tubercle bacilli growing in cells of the guinea pig omentum five days after inoculation. The large, irregularly oval structures are nuclei of omental cells. Their cytoplasm cannot be seen in this photomicrograph. At the upper edge of the preparation is the dark nucleus of a phagocytic cell. (× 1500)

and large mononuclear cells at twenty-four hours. The preparations made on the fifth day showed numerous groups of "freely-growing bacilli" (11) in the omentum of the control pig (figure 3). Relatively few such bacilli could be found in the omentum of the streptomycin-treated pig.

By the end of the fifth week after inoculation, 7 of the control pigs had died; the remainder were sacrificed. All of them showed marked thickening and matting of the omentum. None of the streptomycin-treated pigs died. They were sacrificed at intervals, one being allowed to live for ten weeks. None of them showed advanced disease in the omentum.

In the hope of obtaining a more pronounced difference between control and

[5] It is evident that the omental index of any given animal will vary slightly depending upon whether or not it has a full stomach. In the experiments here reported the animals were allowed to eat lettuce trimmings *ad libitum* on the morning of the day they were sacrificed.

treated pigs, 10 animals were inoculated intraperitoneally with five times the standard dose, namely, 0.5 mg. of virulent tubercle bacilli suspended in 0.5 ml. of saline. At the same time the therapeutic dose of streptomycin was reduced to 20 mg. per kg. daily per animal. At the end of fourteen days the omentum was

TABLE 1

THE THERAPEUTIC EFFECT OF STREPTOMYCIN AND OF ISONIAZID AS REVEALED BY THE OMENTAL INDEX

ANIMAL NUMBER	TIME SINCE INOCULATION	THERAPY	WEIGHT OF PIG	WEIGHT OF OMENTUM	OMENTAL INDEX	
					Individual	Average
			kg.	*gm.*		
1	2 weeks	Infected control	0.290	1.86	6.06	
2	2 weeks	Infected control	0.300	1.51	5.03	
3	2 weeks	Infected control	0.325	1.33	4.09	
4	2 weeks	Infected control	0.290	2.16	7.41	5.65
5	2 weeks	Streptomycin (40 mg. per kg.)	0.280	0.19	0.68	
6	2 weeks	Streptomycin (40 mg. per kg.)	0.280	0.27	0.96	
7	2 weeks	Streptomycin (40 mg. per kg.)	0.280	0.25	0.89	
8	2 weeks	Streptomycin (40 mg. per kg.)	0.330	0.24	0.73	0.81
9	2 weeks	Isoniazid (10 mg. per kg.)	0.320	0.22	0.69	
10	2 weeks	Isoniazid (10 mg. per kg.)	0.280	0.19	0.66	
11	2 weeks	Isoniazid (10 mg. per kg.)	0.300	0.21	0.70	
12	2 weeks	Isoniazid (10 mg. per kg.)	0.290	0.24	0.83	0.72
13	2 weeks	Isoniazid (3 mg. per kg.)	0.320	0.20	0.63	
14	2 weeks	Isoniazid (3 mg. per kg.)	0.260	0.18	0.69	
15	2 weeks	Isoniazid (3 mg. per kg.)	0.320	0.25	0.78	
16	2 weeks	Isoniazid (3 mg. per kg.)	0.280	0.16	0.57	0.67
17	3 weeks	Infected control	0.290	3.03	10.44	
18	3 weeks	Infected control	0.290	2.56	8.82	9.63
19	3 weeks	Streptomycin (40 mg. per kg.)	0.290	0.19	0.66	
20	3 weeks	Streptomycin (40 mg. per kg.)	0.260	0.24	0.92	0.79
21	3 weeks	Isoniazid (10 mg. per kg.)	0.260	0.17	0.65	
22	3 weeks	Isoniazid (10 mg. per kg.)	0.300	0.16	0.53	0.59
23	3 weeks	Isoniazid (3 mg. per kg.)	0.290	0.16	0.55	
24	3 weeks	Isoniazid (3 mg. per kg.)	0.300	0.10	0.33	0.44

found markedly swollen and matted in both the treated and the control animals. From this it was assumed that the therapeutic capacity of the smaller daily dose of streptomycin had been exceeded by the large (0.5 mg.) dose of tubercle bacilli given to each animal.

Effect of streptomycin in comparison with isoniazid: Isoniazid in solution[6]

[6] Nydrazid obtained from E. R. Squibb & Sons, New York.

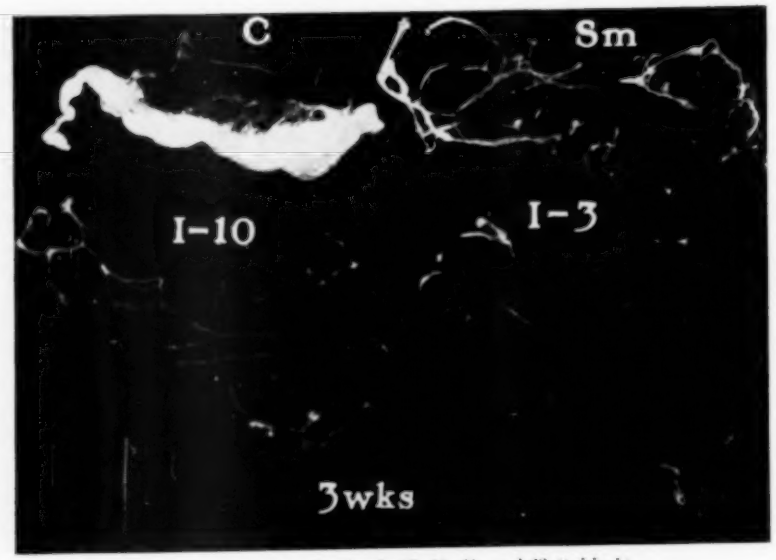

FIG. 4. Omenta of animals 17, 19, 21, and 23 (table 1)

TABLE 2

THE THERAPEUTIC EFFECT OF DIFFERENT DOSES OF ISONIAZID AS REVEALED BY THE OMENTAL INDEX

ANIMAL NUMBER	TIME SINCE INOCULATION	THERAPY	WEIGHT OF PIG	WEIGHT OF OMENTUM	OMENTAL INDEX	
					Individual	Average
			kg.	*gm.*		
1	2 weeks	Infected control	0.240	1.78	7.41	
2	2 weeks	Infected control	0.270	1.68	6.22	
3	2 weeks	Infected control	0.220	1.34	6.09	
4	2 weeks	Infected control	0.220	1.43	6.50	
5	2 weeks	Infected control	0.245	2.06	8.40	6.92
6	2 weeks	Isoniazid (10 mg. per kg.)	0.210	0.24	1.14	
7	2 weeks	Isoniazid (10 mg. per kg.)	0.290	0.27	0.93	
8	2 weeks	Isoniazid (10 mg. per kg.)	0.270	0.21	0.74	
9	2 weeks	Isoniazid (10 mg. per kg.)	0.250	0.15	0.60	
10	2 weeks	Isoniazid (10 mg. per kg.)	0.250	0.21	0.84	0.85
11	2 weeks	Isoniazid (3 mg. per kg.)	0.235	0.14	0.59	
12	2 weeks	Isoniazid (3 mg. per kg.)	0.290	0.28	0.96	
13	2 weeks	Isoniazid (3 mg. per kg.)	0.220	0.19	0.86	
14	2 weeks	Isoniazid (3 mg. per kg.)	0.295	0.31	1.05	
15	2 weeks	Isoniazid (3 mg. per kg.)	0.210	0.14	0.66	0.82

was available for the third experimental series. Thirty-six guinea pigs inoculated intraperitoneally with 0.1 mg. of tubercle bacilli were divided into four groups,

three of the groups to be given therapy as indicated in table 1. In order to study histologically any changes in the omentum, one animal from each group was sacrificed at forty-eight hours and 2 from each group after one week. Omental spreads were made and stained. As might be expected from the work with cell cultures (6, 9), the omental spreads showed greater supression of bacillary growth by isoniazid than by streptomycin.

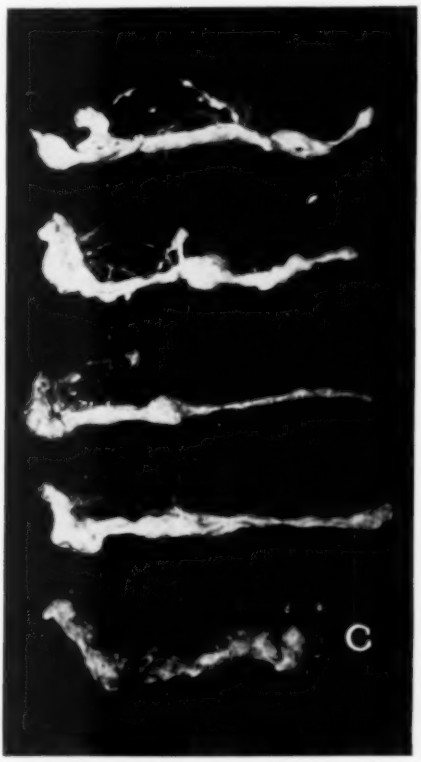

FIG. 5. Omenta of 5 infected control animals (table 2)

Of the 6 remaining pigs in each group, 4 were sacrificed at the end of the second week and 2 at the end of the third week. The results are summarized in table 1. In figure 4 may be seen the omenta of animals 17, 19, 21, and 23.

The lungs, liver, and spleen of each animal were examined also. At the end of two weeks, little difference was noted between the organs of the treated and the control animals except that the spleens of the latter were approximately twice the size of those of the animals which had received chemotherapy.

Comparison of different doses of isoniazid: In order to check the results obtained in the preceding experiment, a final series of 15 pigs was inoculated in the usual

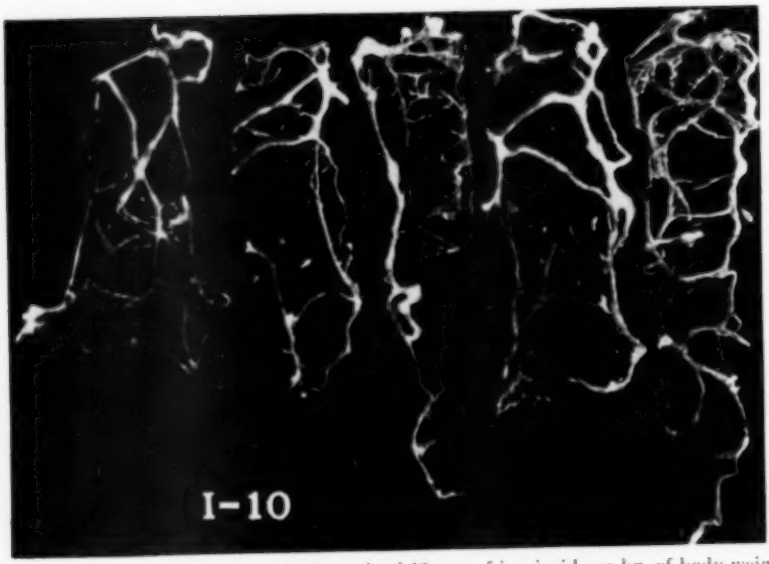

FIG. 6. Omenta of 5 animals which received 10 mg. of isoniazid per kg. of body weight (table 2).

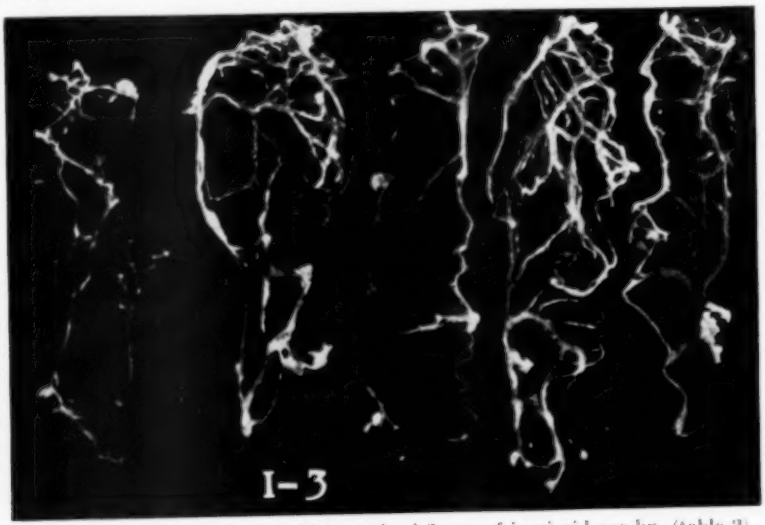

FIG. 7. Omenta of 5 animals which received 3 mg. of isoniazid per kg. (table 2)

manner. The pigs were divided into three groups, two of the groups being given daily injections of isoniazid as indicated in table 2. The results are shown in table 2 and illustrated in figures 5 through 7.

With regard to capacity to suppress the growth of tubercle bacilli, no significant difference was noted between the 3 mg. and 10 mg. doses of isoniazid.

DISCUSSION

The most essential factor in the successful use of the omental index is that the inoculation of tubercle bacilli should actually be made into the peritoneal cavity. If this is done, using a dose of 0.1 mg. or more of virulent microorganisms, the omentum of the animal untreated with antimicrobials will always be found (after two weeks or longer) to be densely matted together and greatly increased in weight. Failure to find the omentum thus diseased can mean only one thing, that the inoculum was lost in the appendix or a loop of intestine. The inoculating technique described earlier in this paper has given uniformly successful intraperitoneal inoculations.

Another essential for the obtaining of maximum sensitivity of the omental index is the exclusion of any extraneous tissue. This is the reason for removing the pancreas before the omentum is weighed even though the gland actually grows out into the omentum adjacent to the spleen (figure 2) and, to one unfamiliar with the anatomy of the guinea pig, might be considered mere perivascular thickening. As the weight of the pancreas in the normal pig is approximately four times the weight of the omentum without the pancreas, however, the inclusion of the gland would greatly decrease the sensitivity of the index. From data given in their paper (14), it is evident that Saz and his associates did include the pancreas in figuring their omental index.

In addition to the study of Saz, the intraperitoneal route of inoculating guinea pigs for the testing of therapeutic agents has been used by Smith and his associates (16) and Dessau and his co-workers (17, 18). Each of the latter two groups of workers, however, allowed the infected guinea pigs to live for many weeks and merely averaged the extent of omental involvement as one factor, along with the extent of involvement of the other organs, in determining the outcome of the experiment.

The results given in table 1 suggest a slight superiorty of isoniazid over streptomycin under the conditions of the experiment. This might have been predicted from the fact that the molecule of isoniazid penetrates cells readily and thus prevents any phagocytosed bacilli from multiplying (6, 9). The large streptomycin molecule, on the other hand, penetrates cells poorly (19), so that phagocytosed bacilli are partially protected from its action.

Mackaness and Smith have indicated in a recent paper (10) that 0.1 γ of isoniazid per ml. of medium is practically as effective as 10 γ in killing phagocytosed bacilli *in vitro*. The results shown in tables 1 and 2 likewise indicate that 3 mg. of isoniazid per kg. of body weight are entirely adequate to suppress the proliferation of tubercle bacilli in the guinea pig.

The omental index, as used in the study of antituberculous agents, shares some of the limitations of the cell culture technique (6, 9), for both give little indication of what might be expected from long-term chemotherapy. Both techniques, on the other hand, since they offer an answer to chemotherapeutic

problems in a matter of days, are particularly suited to the initial screening of antimicrobial agents.

SUMMARY

In searching for a method for the rapid evaluation of antimicrobial agents, guinea pigs were inoculated intraperitoneally with 0.1 mg. of virulent tubercle bacilli. At the end of two weeks the greatly swollen omentum was removed and weighed; the ratio of the weight of the omentum in grams to the weight of the guinea pig in kilograms has been called the omental index. If an effective antimicrobial is used, the omentum increases in weight very little and the omental index remains near the normal level.

Under the experimental conditions described, isoniazid appears slightly more effective than streptomycin. Three milligrams of isoniazid per kg. of body weight are entirely adequate to suppress the proliferation of tubercle bacilli in the guinea pig.

SUMARIO

Uso del Epiplón del Cobayo como Indice de la Eficacia de las Drogas Antimicrobianas en la Tuberculosis Experimental

En busca de un método para la rápida justipreciación de los agentes antimicrobianos, se inoculó a cobayos intraperitonealmente 0.1 mg. de bacilos tuberculosis virulentos. Al cabo de dos semanas, se extirpó y pesó el epiplón, que estaba considerablemente hinchado, la proporción del peso en gramos del epiplón al peso del cobayo en kilogramos ha sido denominada índice epiploico. Si se usa un antimicrobiano eficaz, el epiplón aumenta muy poco de peso y el índice epiploico no se aparta mucho del tenor normal.

En las condiciones de experimentación descritas, la isoniacida parece ser ligeramente más eficaz que la estreptomicina. Tres miligramos de isoniacida por kg. de peso vivo son absolutamente adecuados para suprimir la proliferación de los bacilos tuberculosos en los cobayos.

RESUME

Emploi de l'épiploon de porc comme indice d'efficacité des médicaments bactéricides dans la tuberculose expérimentale

A la recherche d'une méthode permettant une évaluation rapide des agents microbicides, les auteurs ont pratique, chez le cobaye, l'inoculation intrapéritonéale de 0.1 mg. de bacilles tuberculeux virulents. Au bout de deux semaines l'épiploon, qui présentait une tuméfaction considérable, était excisé et pesé; le rapport du poids de l'épiploon au poids des cobayes exprimé en kilogrammes a été dénommé "omental index". Lorsqu'un agent antibactérién efficace est utilisé, le poids l'épiploon augmente très peu et cet "omental index" reste à un niveau normal.

Dans les conditions de l'expérimentation, l'isoniazide paraît légèrement plus efficace que la streptomycine. Trois milligrammes par kg. d'isoniazide de poids sont parfaitement adéquats pour supprimer la prolifération du bacille tuberculeux chez le cobaye.

REFERENCES

(1) Feldman, W. H., Hinshaw, H. C., and Mann, F. C.: Streptomycin in experimental tuberculosis, Am. Rev. Tuberc., 1945, *52*, 269.

(2) Steenken, W., Jr., and Wolinsky, E.: Streptomycin in experimental tuberculosis: II. Response in guinea pigs infected with strains of varying degrees of streptomycin resistance, Am. Rev. Tuberc., 1948, *58*, 353.

(3) Lurie, M. B.: Experimental air-borne tuberculosis and its control, Am. Rev. Tubere., 1947, *55*, 124.

(4) Steenken, W., Jr., Wolinsky, E., and Pratt, P. C.: Streptomycin and PAS in experimental tuberculosis of guinea pigs infected intracerebrally with virulent tubercle bacilli, Am. Rev. Tubere., 1951, *64*, 87.

(5) Youmans, G. P., and McCarter, J. C.: Streptomycin in experimental tuberculosis: Its effect on tuberculous infection in mice produced by *M. tuberculosis*, Am. Rev. Tubere., 1945, *52*, 432.

(6) Suter, E.: Action of streptomycin and isonicotinic acid hydrazide on *M. tuberculosis* within phagocytes, Am. Rev. Tubere., 1952, *65*, 775.

(7) Suter, E.: The multiplication of tubercle bacilli within normal phagocytes in tissue culture, J. Exper. Med., 1952, *96*, 137.

(8) Mackaness, G. B.: The action of drugs on intracellular tubercle bacilli, J. Path. & Bact., 1952, *64*, 429.

(9) Mackaness, G. B., and Smith, N.: The action of isoniazid on intracellular tubercle bacilli, Am. Rev. Tubere., 1952, *66*, 125.

(10) Mackaness, G. B., and Smith, N.: The bactericidal action of isoniazid, streptomycin, and terramycin on extracellular and intracellular tubercle bacilli, Am. Rev. Tubere., 1953, *67*, 322.

(11) Woodruff, C. E.: A free growth period of tubercle bacilli in the guinea pig omentum as related to the hypersensitive state, Am. J. Path., 1934, *10*, 739.

(12) Gardner, L. U.: Studies on tissue reactions to primary infection and reinfection with the tubercle bacillus: I. A histological examination of the omentum and other subperitoneal tissues, Am. Rev. Tubere., 1929, *20*, 201.

(13) Woodruff, C. E., and Kelly, R. G.: Growth of tubercle bacilli in tissues of normal and allergic guinea pigs, Am. Rev. Tubere., 1940, *42*, 782.

(14) Saz, A. K., Johnson, F. R., Burger, A., and Bernheim, F.: Effect of aromatic iodine compounds on the tubercle bacillus, Am. Rev. Tubere., 1943, *48*, 40.

(15) Willis, H. S., and Cummings, M. M.: Diagnostic and Experimental Methods in Tuberculosis, ed. 2, Charles C Thomas, Springfield, Illinois, 1952.

(16) Smith, M. I., Emmart, E. W., and Stohlman, E. F.: The action of some derivatives of 4-4′ diaminodiphenylsulfone in experimental tuberculosis, Am. Rev. Tubere., 1943, *48*, 32.

(17) Dessau, F. I., Yeager, R. L., and Kulish, M.: A simplified guinea pig test for tuberculostatic agents, Am. Rev. Tubere., 1949, *60*, 223.

(18) Dessau, F. I., Yeager, R. L., Burger, F. J., and Williams, J. H.: Pyrazinamide (Aldinamide) in experimental tuberculosis of the guinea pig, Am. Rev. Tubere., 1952, *65*, 519.

(19) Marshall, E. K., Jr.: The absorption, distribution and excretion of streptomycin, J. Pharmacol., 1948, *92*, 43.

SERUM GLYCOPROTEIN CONCENTRATIONS IN EXPERIMENTAL TUBERCULOSIS OF GUINEA PIGS[1, 2]

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INTRODUCTION

The importance of the serum polysaccharide concentrations in clinical and in experimental tuberculosis has been demonstrated by the investigations of Seibert and her associates (1, 2, 3, 4). Statistically significant increases were reported in patients with moderately advanced and far advanced tuberculosis when compared with the values from a control group of normal persons (2, 3).

Based on conclusions drawn from a study of experimental tuberculosis in rabbits, it has been suggested that serum polysaccharide concentrations might be of prognostic value (4). Turner and co-workers found the mucoprotein fraction of serum to be significantly elevated in far advanced pulmonary tuberculosis (5). Extremely high values were associated with a poor prognosis.

Although the guinea pig is widely employed in studies of experimental tuberculosis, apparently no data are available concerning the effects of the disease process on the protein-bound carbohydrates of serum of the species. In the course of investigating the post-treatment effects of corticotropin and of cortisone on experimental tuberculosis (6), the opportunity was presented to study the glycoproteins of guinea pig serum. This report is concerned with the effects of experimental tuberculosis and various treatment regimens on the total serum polysaccharide, mucoprotein polysaccharide, and total serum protein concentrations of the guinea pig.

MATERIALS AND METHODS

Definitions: Because there is no generally accepted nomenclature for the glycoproteins of serum, it is advisable to define the terms employed.

> *Glycoproteins:* Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid (7).
> *Total serum polysaccharide:* The non-glucosamine polysaccharide component or hexose moiety of the serum glycoproteins precipitated by an ethanol concentration of 90 per cent by volume at 25°C.
> *Serum mucoprotein polysaccharide:* The non-glucosamine polysaccharide component or hexose moiety of the perchloric acid-soluble, phosphotungstic acid-insoluble proteins occurring in serum. (Serum mucoprotein polysaccharide is a constituent of the total serum polysaccharide.)

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[2] This investigation was supported, in part, by a research grant from the National Microbiological Institute of the National Institutes of Health, Public Health Service.

One hundred and seventy-five guinea pigs with an average weight of 645 gm. were found to be nonreactors to the intracutaneous injection of 0.1 ml. of a 5.0 per cent solution of Old Tuberculin. The animals were inoculated subcutaneously in the inguinal region with 1.0 ml. of Dubos medium, containing approximately 60,000 organisms of the BSS strain of *Mycobacterium tuberculosis* var. *hominis*.[3] Four weeks after inoculation, treatment was initiated as outlined in tables 1 and 2 and was continued for twenty-one days. The guinea pigs were allowed to survive sixty-eight days after the termination of treatment and were then sacrificed by exsanguination from the heart and air embolism. The serum was separated and stored in the frozen state until the determinations could be conducted. Blood was also obtained by cardiac puncture from 27 normal animals and treated in a similar manner.

Chemical Determinations

Serum mucoprotein polysaccharide: Serum mucoprotein polysaccharide was determined by a modification of the procedure of Winzler and associates (8). Since the modification possesses advantages over the original method with regard to economy of serum, better reproducibility, ease of filtration, reduction of co-precipitation, and elimination of the generation of carbon dioxide, it is reported in detail.

The following reagents were used:

0.85 per cent sodium chloride
1.8 M. perchloric acid
5 per cent phosphotungstic acid in 2 N. hydrochloric acid
absolute ethanol
0.1 N. sodium hydroxide
21.6 N. sulfuric acid
2.0 per cent orcinol (Eastman Kodak) in 21.6 N. sulfuric acid

One volume of serum was diluted with nine volumes of 0.85 per cent sodium chloride. To 5.0 ml. of the diluted serum were added dropwise 2.5 ml. of 1.8 M. perchloric acid. The contents were mixed by inversion. In exactly ten minutes the precipitated proteins were filtered through Whatman No. 50 filter paper (7.0 cm.). To 5.0 ml. of the perchloric acid filtrate was added 1.0 ml. of the phosphotungstic acid reagent and the contents were mixed by agitation. After ten minutes the precipitated mucoprotein was centrifuged at 2,000 r.p.m. for ten minutes, washed once with 10.0 ml. of absolute ethanol, and recentrifuged. The ethanol was decanted and the precipitate was allowed to drain for five minutes by inversion of the tube. One ml. of 0.1 N. sodium hydroxide was added, and the precipitate was agitated until dissolved. The non-glucosamine polysaccharide in the mucoprotein solution was determined as follows: 7.5 ml. of 21.6 N. sulfuric acid were added to the solution followed by 1.0 ml. orcinol reagent. The contents of the tube were thoroughly mixed and placed in a water bath at 80° C. and shielded from direct lighting. The tubes were kept in the water bath for fifteen minutes, then cooled in the dark for the same period of time in an ice bath. Standards containing 0.1 mg. and 0.2 mg. of an equimolar mixture of galactose and mannose and a reagent blank were treated in a similar manner. The non-glucosamine polysaccharide content was then determined from Klett-Summerson photoelectric colorimeter readings, employing the No. 54 (green) filter.

Total serum polysaccharide: Total serum polysaccharide was determined by a modification of the procedure of Lustig and Langer (9). One volume of serum was diluted with nine volumes of 0.85 per cent sodium chloride. One milliliter of the diluted serum was added dropwise to 10 ml. of absolute ethanol contained in a 15 ml. conical centrifuge tube. The contents were mixed by inversion and allowed to stand for ten minutes. The precipitated proteins were centrifuged at 2,000 r.p.m. for ten minutes, washed with 10 ml. of absolute ethanol, and recentrifuged. The ethanol was decanted and the precipitate drained by in-

[3] Obtained through the courtesy of Dr. Emil Bogen. This strain was originally isolated from a patient with active pulmonary tuberculosis by Dr. C. Richard Smith, Barlow Sanatorium, Los Angeles, California.

version of the tube. One milliliter of distilled water was added and the concentration of the total non-glucosamine polysaccharide was determined in the same manner as for mucoprotein polysaccharide, beginning with the addition of 7.5 ml. of 21.6 N. sulfuric acid followed by 1.0 ml. of orcinol reagent.

Total serum protein: Total serum protein was determined by the biuret method of Weichselbaum (10).

Analysis of results: As it is impractical to include the actual analyses of all animals, the mean, standard error of the mean, and probability values are included in tables 1 and 2. The significance of the deviations from the normal mean was determined by the *t* test of Fisher (11).

RESULTS

The summaries of the treatment regimens, average indices of tuberculosis, results of chemical analyses, and other pertinent statistical data are presented in tables 1 and 2.

Total serum polysaccharide: The mean value for the polysaccharide of the ethanol-precipitated, total serum proteins in normal serum was 111 mg. per 100 ml. (table 1). The content of serum polysaccharide was significantly elevated in all groups with tuberculosis. The small range of the mean values among the infected groups, 176 to 190 mg. per 100 ml., suggested that the increased serum concentrations of polysaccharide were due to the disease process and were not the result of the treatment regimens.

Serum mucoprotein polysaccharide: An average value of 29 mg. per 100 ml. was obtained for the normal guinea pigs of *Group A* (table 1). All infected groups exhibited an increased serum mucoprotein polysaccharide content of statistical significance. The limited range of the mean values for the groups with tuberculosis, 54 to 69 mg. per 100 ml., suggested that the elevated mucoprotein polysaccharide concentrations were a result of the experimental infection and that prior treatment was not a factor.

Relationship of serum mucoprotein polysaccharide to total serum polysaccharide: The quantitative relations existing between serum mucoprotein polysaccharide and total serum polysaccharide have never been elucidated. A sufficient number of determinations have been made in the present investigation to make the results of the computations of interest to workers in the field of serum glycoproteins. Mucoprotein polysaccharide comprised 26.1 per cent of the total serum polysaccharide in the normal group, *Group A* (table 1). A significant increase in percentage occurred in all infected groups with the exceptions of *Group B*, untreated, and *Group F1*, treated with the higher dosage of streptomycin. The values within each group were subject to some variability, as indicated by the magnitude of the standard error of the mean. Mucoprotein polysaccharide comprised a greater percentage of the total serum polysaccharide in the guinea pig than in man (*cf.* 13).

Total serum protein: A mean value of 5.4 gm. per 100 ml. was obtained for the sera of 27 normal guinea pigs (table 2). A significant hyperproteinemia occurred in all infected groups irrespective of the treatment regimen. Two of the groups which received corticotropin, *C1* and *C3*, had significantly higher serum protein concentrations than the infected, untreated animals of *Group B*. The observa-

TABLE 1

SUMMARY OF SERUM POLYSACCHARIDE DETERMINATIONS

GROUP	NUMBER OF ANIMALS	TREATMENT	AVERAGE* INDEX OF TUBERCULOSIS	TOTAL SERUM POLYSACCHARIDE		MUCOPROTEIN POLYSACCHARIDE		MUCOPROTEIN POLYSACCHARIDE ÷ TOTAL SERUM POLYSACCHARIDE × 100	
				Mean†	*P*	Mean†	*P*	Mean†	*P*
				mg. per 100 ml.		*mg. per 100 ml.*		*per cent*	
A, Normal	27	None	0.0	111 ± 2.5		29 ± 1.1		26.1 ± 0.79	
B, Infected	12	None	9.3	190 ± 4.6	<0.01	56 ± 2.7	<0.01	29.5 ± 1.26	<0.05
C1, Infected	17	0.1 mg. of corticotropin per day	9.8	176 ± 4.5	<0.01	56 ± 2.3	<0.01	31.8 ± 0.85	<0.01
C2, Infected	13	1.0 mg. of corticotropin per day	9.2	181 ± 4.6	<0.01	57 ± 2.7	<0.01	31.5 ± 1.06	<0.01
C3, Infected	12	10.0 mg. of corticotropin per day	9.7	191 ± 3.4	<0.01	60 ± 3.0	<0.01	31.4 ± 1.24	<0.01
D1, Infected	17	0.2 mg. of cortisone per day	8.6	181 ± 4.3	<0.01	56 ± 2.0	<0.01	30.9 ± 0.94	<0.01
D2, Infected	15	2.0 mg. of cortisone per day	9.7	179 ± 4.4	<0.01	60 ± 2.2	<0.01	33.5 ± 0.87	<0.01
D3, Infected	12	20.0 mg. of cortisone per day	10.3	176 ± 5.6	<0.01	57 ± 2.9	<0.01	32.4 ± 1.03	<0.01
E, Infected	6	10.0 mg. of beryllium chloride 3 times weekly	13.2	183 ± 7.0	<0.01	69 ± 2.9	<0.01	37.8 ± 2.08	<0.01
F1, Infected	10	30.0 mg. of streptomycin per day	5.5	179 ± 4.3	<0.01	54 ± 2.3	<0.01	30.2 ± 1.22	<0.02
F2, Infected	9	30.0 mg. of streptomycin once a week	8.7	188 ± 6.0	<0.01	60 ± 2.4	<0.01	31.9 ± 0.71	<0.01

* Each organ (spleen, liver, lungs, lymph nodes) was assigned a value from 0 to 4 according to the amount of macroscopic tuberculous disease. Maximum total for each animal was 16. The average index of tuberculosis was the average total for each group (12).

† Including the standard error of the mean calculated from the relation

$$E_m = [\Sigma d^2/(N(N-1))]^{\frac{1}{2}}$$

where d is the deviation from the mean and N is the number of observations.

TABLE 2

THE RELATIONSHIPS OF THE SERUM POLYSACCHARIDES TO THE TOTAL SERUM PROTEIN

GROUP	NUMBER OF ANIMALS	TREATMENT	AVERAGE* INDEX OF TUBERCULOSIS	TOTAL SERUM PROTEIN		TOTAL SERUM POLYSACCHARIDE ÷ TOTAL SERUM PROTEIN × 100		MUCOPROTEIN POLYSACCHARIDE÷TOTAL SERUM PROTEIN × 100	
				Mean†	P	Mean†	P	Mean†	P
				gm. per 100 ml.		*per cent*		*per cent*	
A, Normal	27	None	0.0	5.4 ± 0.08		2.1 ± 0.05		0.5 ± 0.03	
B, Infected	12	None	9.3	5.9 ± 0.13	<0.01	3.2 ± 0.08	<0.01	1.0 ± 0.06	<0.01
C1, Infected	17	0.1 mg. of corticotropin per day	9.8	6.6 ± 0.09	<0.01	2.7 ± 0.05	<0.01	0.9 ± 0.04	<0.01
C2, Infected	13	1.0 mg. of corticotropin per day	9.2	6.3 ± 0.10	<0.01	2.9 ± 0.06	<0.01	0.9 ± 0.05	<0.01
C3, Infected	12	10.0 mg. of corticotropin per day	9.7	6.6 ± 0.12	<0.01	2.9 ± 0.09	<0.01	0.9 ± 0.06	<0.01
D1, Infected	17	0.2 mg. of cortisone per day	8.6	6.3 ± 0.10	<0.01	2.9 ± 0.06	<0.01	0.9 ± 0.03	<0.01
D2, Infected	15	2.0 mg. of cortisone per day	9.7	6.3 ± 0.12	<0.01	2.8 ± 0.08	<0.01	1.0 ± 0.05	<0.01
D3, Infected	12	20.0 mg. of cortisone per day	10.3	6.2 ± 0.15	<0.01	2.8 ± 0.06	<0.01	0.9 ± 0.05	<0.01
E, Infected	6	10.0 mg. of beryllium chloride 3 times weekly	13.2	6.3 ± 0.28	<0.01	2.9 ± 0.10	<0.01	1.1 ± 0.06	<0.01
F1, Infected	10	30.0 mg. of streptomycin per day	5.5	6.2 ± 0.08	<0.01	2.9 ± 0.06	<0.01	0.9 ± 0.04	<0.01
F2, Infected	9	30.0 mg. of streptomycin once a week	8.7	6.2 ± 0.13	<0.01	3.0 ± 0.08	<0.01	1.0 ± 0.04	<0.01

Symbols as in table 1.

tion was in accord with the results of Malkiel (14), who found an increase in the total serum proteins of guinea pigs administered corticotropin and subjected to anaphylactic shock.

Percentage of polysaccharide in total serum protein: Polysaccharide was computed to comprise 2.1 per cent of the total serum proteins of *Group A* (table 2). Higher percentages, of statistical significance, were calculated for all groups with tuberculosis. The percentage of serum glycoproteins in the total serum proteins is much larger since only the non-glucosamine polysaccharide moiety of the glycoprotein molecules was determined. It was evident from the results of the computations that the elevation of the serum polysaccharide did not merely reflect the general elevation in total serum proteins, but that there was a marked increase in carbohydrate-containing proteins.

Percentage of mucoprotein polysaccharide in total serum protein: Mucoprotein polysaccharide was found to comprise an average of 0.5 per cent of the total serum proteins of 27 normal guinea pigs (table 2). A significant increase occurred in all groups with tuberculosis. As in the case of the total serum polysaccharide, the calculated value represents only the hexose component of the conjugated protein. The increased percentages determined for the infected groups indicate both a relative and an absolute increase of serum mucoprotein in comparison with the total serum protein.

DISCUSSION

Previous investigations have shown that the concentration of total serum polysaccharide was increased in such diverse conditions as clinical (1, 2, 3, 5, 9) tuberculosis and experimental tuberculosis of rabbits (4), clinical (2, 3, 13, 15) and experimental (16) carcinoma, late pregnancy and parturition (17), experimental thermal injury (18), and fractures (19). Increased serum mucoprotein concentrations have been reported in clinical tuberculosis (5), clinical (13, 20) and experimental carcinoma (21), rheumatic fever (22), myocardial infarction (23), experimental scurvy (24), immunization (25), experimental endocarditis (25), and following the administration of parathyroid extract (26). The present investigation has extended the observations to experimental tuberculosis of guinea pigs.

Statistically significant increases in the mean values for total serum polysaccharide, mucoprotein polysaccharide, and total serum protein were found for all infected groups when compared with the means of the normal control group.

The increase in serum glycoprotein paralleled to a certain extent the amount of macroscopic tuberculosis. Only *Group E*, which received beryllium chloride, and *Group F1*, treated with streptomycin, exhibited a significant difference in the average index of tuberculosis when compared with the infected, untreated animals of *Group B* (6). The small range in the mean values for total serum polysaccharide and mucoprotein polysaccharide among the infected groups has already been stressed. The average indices of tuberculosis of all infected groups would place them in the far advanced, active, tuberculosis category as classified by clinical criteria (27). Thus, it was apparent that the elevation of the

serum glycoprotein in experimental tuberculosis of guinea pigs was similar to the elevation noted in man as a part of the response to infection (*cf.* 2, 3).

The uniform occurrence of hyperproteinemia in the infected groups suggested that a species difference might occur with regard to the total serum protein response to tuberculosis. Gutman (28), in a summary of clinical investigations, reported that total serum protein concentrations tended to remain within normal limits although occasional moderate hypoproteinemia or hyperproteinemia was noted. The possibility also exists that the elevated serum protein of the inoculated guinea pig may reflect a difference between the acute experimental infection of a susceptible animal and the chronic disease in relatively resistant man.

Treatment with three dosage levels of corticotropin[4] and of cortisone[5], two dosages of streptomycin, and beryllium chloride apparently had little or no effect on the serum glycoprotein values. It may not be inferred from the data, however, that the above substances would not have an effect on serum values. Sixty-eight days elapsed from the termination of treatment to autopsy, and the effect of any agent might well have been dissipated during that period. An investigation of the effects of corticotropin and of cortisone administration on the serum glycoprotein concentrations of normal guinea pigs is in progress.

SUMMARY

Total polysaccharide, mucoprotein polysaccharide, and total protein have been determined in the serum of guinea pigs infected with the B88 strain of *Mycobacterium tuberculosis* var. *hominis* and treated with three graduated dosages of corticotropin (ACTH) and of cortisone, two dosages of streptomycin, and beryllium chloride. Statistically significant increases were found in the total serum polysaccharide, mucoprotein polysaccharide, and total serum protein concentrations of all infected groups, irrespective of the treatment regimen, in comparison with the values in normal control animals.

An improved procedure for the estimation of serum mucoprotein has been presented.

SUMARIO

Las Concentraciones de Glucoproteína Sérica en la Tuberculosis Experimental del Cobayo

Determináronse el polisacárido total, el polisacárido de mucoproteína y la proteían total en el suero de cobayos infectados con la cepa B88 del *Mycobacterium tuberculosis* var. *hominis* y tratados con tres esquemas posológicos graduados de corticotropina y de cortisona, dos esquemas de estreptomicina y cloruro de berilio. De cubriéronse aumentos de importancia estadística en las concentraciones de polisacárido sérico total, polisacárido de proteína y proteína sérica total en todos los grupos infectados, independientemente del régimen terapéutico, comparado con los testigos normales.

Preséntase un procedimiento perfeccionado para el cálculo de la mucoproteína sérica.

[4] The corticotropin (Actrope) used in this investigation was obtained through the courtesy of Mr. Richard C. Bruner and Mr. S. S. Kingman, United Laboratories Ltd., Pasadena, California. One milligram of Actrope is equivalent to one U. S. P. unit.

[5] A large part of the cortisone acetate (Cortone) employed in this investigation was obtained through the courtesy of Dr. J. M. Carlisle, Merck & Co., Rahway, New Jersey.

RESUME

Concentrations de la glycoprotéine sérique dans la tuberculose expérimentale du cobaye

La détermination des concentrations de polysaccharide total, de polysaccharide-mucoprotéine et de protéine totale a été effectuée dans le sérum de cobayes infectés avec la souche B88 du *Mycobacterium tuberculosis* var. *hominis*. Les cobayes ont ensuite été traités sous la forme de trois cures graduées de corticotrophine (ACTH) et de cortisone, deux cures de streptomycine et de chlorure de béryllium. Par comparaison avec les animaux-témoins sains, des augmentations significatives, du point de vue statistique, ont été observées dans les concentrations sériques de polysaccharide total, de polysaccharide-mucoprotéine et de protéine totale dans tous les groupes infectés, quelle qu'ait été la posologie adoptée.

Les auteurs présentent un procédé amelioré pour l'évaluation de la mucoprotéine sérique.

REFERENCES

(1) Seibert, F. B., Nelson, J. W., and Seibert, M. V.: Correlation of extent of tuberculosis with amount of polysaccharide in the serum, Proc. Soc. Exper. Biol. & Med., 1943, *52*, 219.

(2) Seibert, F. B., Seibert, M. V., Atno, A. J., and Campbell, H. W.: Variation in protein and polysaccharide content of sera in the chronic diseases, tuberculosis, sarcoidosis, and carcinoma, J. Clin. Investigation, 1947, *26*, 90.

(3) Seibert, F. B., Pfaff, M. L., and Seibert, M. V.: A serum polysaccharide in tuberculosis and carcinoma, Arch. Biochem., 1948, *18*, 279.

(4) Seibert, F. B., and Seibert, M. V.: Significance of changes in the content of serum polysaccharides during sensitization and development of tuberculosis, Am. Rev. Tuberc., 1950, *62*, 67.

(5) Turner, G. C., Schaffner, F., Eshbaugh, D. E., and Huerga, J. de la: Serum mucoproteins in pulmonary tuberculosis, Dis. of Chest, 1953, *23*, 154.

(6) Weimer, H. E., Boak, R. A., Bogen, E., Drusch, H. E., Miller, J. N., Moshin, J. R., and Carpenter, C. M.: Comparative effects of corticotropin and cortisone on experimental tuberculosis, Am. Rev. Tuberc., 1953, *68*, 31.

(7) Committee on Protein Nomenclature: Joint recommendations of the physiological and biochemical committees on protein nomenclature, J. Biol. Chem., 1908, *4*, viii.

(8) Winzler, R. J., Devor, A., Mehl, J. W., and Smyth, I.: Studies on the mucoproteins of human plasma: I. Determination and isolation, J. Clin. Investigation, 1948, *27*, 609.

(9) Lustig, B., and Langer, A.: Über die bestimmung und den gehalt an freiem, eiweiss— und gebundenem nichteiweisszucker in normalen und pathologischen serum, liquor, und harn, Biochem. Ztschr., 1931, *242*, 320.

(10) Weichselbaum, T. E.: An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma, Am. J. Clin. Path., 1946, *16*, (Tech. Sec. 40).

(11) Snedecor, G. W.: Statistical Methods, ed. 4, The Iowa State College Press, Ames, Iowa, 1946.

(12) Bogen, E.: Experimental tuberculosis in guinea pigs, Nat. Tuberc. A. Tr., 1932, p. 1.

(13) Greenspan, E. M., Lehman, I., Graff, M. M., and Schoenbach, E. B.: A comparative study of the serum glycoproteins in patients with parenchymatous hepatic disease or metastatic neoplasia, Cancer, 1951, *4*, 972.

(14) Malkiel, S.: Biochemical studies on blood of guinea pigs treated with pituitary adrenocorticotrophic hormone and cortisone, Proc. Soc. Exper. Biol. & Med., 1951, *77*, 333.

(15) Shetlar, M. R., Foster, J. V., Kelly, K. H., Shetlar, C. L., Bryan, R. S., and Everett, M. R.: The serum polysaccharide level in malignancy and in other pathological conditions, Cancer Research, 1950, *9*, 515.

(16) Shetlar, M. R., Erwin, C. P., and Everett, M. R.: Serum polysaccharide levels in rats bearing the Walker 256 tumor, Cancer Research, 1950, *10*, 445.

(17) Shetlar, M. R , Kelly, K. H., Foster, J. V., Shetlar, C. L., and Everett, M. R.: Serum polysaccharide levels in pregnancy, parturition, and the postpartum state, Am. J. Obst. & Gynec., 1950, *59*, 1140.

(18) Knobloch, W. H., Jr., Nagle, P., Shetlar, C. L., and Shetlar, M. R.: Effect of epidermal damage upon serum polysaccharides, Proc. Soc. Exper. Biol. & Med., 1952, *81*, 417.

(19) Keyser, J. W.: Blood serum protein-bound carbohydrates after injury, J. Clin. Path., 1952, *5*, 194.

(20) Winzler, R. J., and Smyth, I.: Studies on the mucoproteins of human plasma: II. Plasma mucoprotein levels in cancer patients, J. Clin. Investigation, 1948, *27*, 617.

(21) Catchpole, H. R.: Serum and tissue glycoproteins in mice bearing transplantable tumors, Proc. Soc. Exper. Biol. & Med., 1950, *75*, 224.

(22) Kelly, V. C., Good, R. A., and McQuarrie, I.: Serum mucoproteins in children in health and disease with special reference to rheumatic fever, Pediatrics, 1950, *5*, 824.

(23) Simkin, B., Bergman, H. C., and Prinzmetal, M.: Studies on coronary circulation: V. Quantitative changes in a serum mucoprotein following the occurrence of myocardial infarction, Am. J. Med., 1949, *6*, 734.

(24) Pirani, C. L., and Catchpole, H. R.: Serum glycoproteins in experimental scurvy, Arch. Path., 1951, *51*, 597.

(25) Hammerstrom, R. N., Adams, F. H., Bussman, J., and Lillehei, C. W.: Normal mucoprotein values in the dog: Response to distemper vaccine and to endocarditis, Proc. Soc. Exper. Biol. & Med., 1952, *79*, 257.

(26) Engel, M. B.: Mobilization of mucoprotein by parathyroid extract, Arch. Path., 1952, *53*, 339.

(27) Diagnostic Standards and Classification of Tuberculosis, 1950 edition, National Tuberculosis Association, New York.

(28) Gutman, A. B.: The plasma proteins in disease, *Advances in Protein Chemistry*, Academic Press Inc., New York, 1948, *4*, 212.

Case Reports

DIFFUSE INTERSTITIAL FIBROSIS OF THE LUNGS[1]

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INTRODUCTION

During the past fifty years, occasional reports have appeared of patients who present progressive interstitial pulmonary fibrosis and a clinical picture of progressive dyspnea and cyanosis, which gradually leads to asphyxiation and death. These reports have been scattered and, in general, the disease has been termed idiopathic. In 1933 and 1935, Hamman and Rich described two such cases and, by 1944, they had collected a total of four cases. They termed the disease *diffuse interstitial fibrosis of the lungs* and their reports are the first systematic description of the disease entity.

Characteristically, the onset is acute and the disease is rapidly progressive, showing a picture of dyspnea, cyanosis, and chronic asphyxiation gradually leading to death. Frequently, there is evidence of cor pulmonale with right-sided heart failure. At autopsy, the lungs are found to be the site of widespread connective tissue hyperplasia throughout the interstitial structures. The alveolar walls are tremendously thickened and hyalinized. There is little or no infiltration or inflammatory exudate within the alveoli themselves and there is a marked absence of stainable bacteria within the alveoli.

In three of the four originally reported cases, eosinophils were present in the interstitial tissue. Subsequently, fifteen other cases have been reported, making a total of nineteen. The etiology of the syndrome is still unknown, although it has been theorized that the pulmonary changes might represent a diffuse reaction to some viral infection. As more information has been accumulated about the disease syndrome, it has become obvious that the term acute is not an acceptable one for all such cases. Several cases have been reported in which the periods of observation have been as long as fifteen months to three years.

The present report is prompted by the writer's observation during the past three years of two patients who fulfilled the criteria for this disease syndrome. These patients presented several unusual features which differed from the preceding reports. Heretofore, only two patients have been reported as being treated with corticotropin or cortisone.

CASE REPORTS

Case 1. J. L. P., a thirty-one-year-old farmer, was admitted in October, 1952, with the history of an acute onset of fever, anterior chest pain, and a persistent cough of three months' duration. This illness began following an upper respiratory infection and progressed despite the use of penicillin and streptomycin. He was seen at a local hospital and was treated with aureomycin without any real relief. Subsequently, he was transferred to Charity Hospital at New Orleans, Louisiana, where he remained approximately one month. During that time, an extensive examination failed to reveal the etiology of the patient's cough, dyspnea, chest pain, and low-grade fever. His chest roentgenograms at that institu-

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tion consistently showed a bilateral, interstitial type of infiltration which failed to change under therapy. While at that hospital he received penicillin and streptomycin, with only slight symptomatic relief. He was then discharged from that institution and returned home. Three days after his discharge, his dyspnea became so severe that he sought care again and was admitted to the Veterans Administration Hospital, New Orleans, Louisiana, in October, 1952. On admission there, his main complaints were dyspnea and malaise.

Physical examination: The patient was an asthenic, adult white male who appeared acutely ill due to dyspnea. Marked cyanosis was obvious. His rectal temperature was 103°F. The respiratory rate was approximately 40 per minute and was rather shallow. The cyanosis cleared considerably in an oxygen tent, but minimal cyanosis of the nail beds was still present. The right posterior pharynx showed evidence of inflammation and injection, but no ulceration or membrane. Coarse breath sounds could be heard diffusely in the lungs, most marked at both bases. At the right base posteriorly, many moist rales were heard, most frequently at the end of inspiration. Generalized lymphadenopathy was noted in the cervical, inguinal, and axillary areas. These lymph nodes were small, soft, and nontender. The chest roentgenogram revealed a marked, generalized interstitial type of infiltration which was greatest in the mid-lung fields bilaterally (figure 1). A flat roentgenogram of the abdomen revealed moderate splenomegaly. There was a slight leukocytosis of 12,000, with a normal differential count. On admission, chemical studies of the blood revealed a moderate increase in the carbon dioxide combining power. An initial electrocardiogram was interpreted as being within normal limits. After the collection of sputum for initial bacteriologic studies, the patient was given terramycin with no real symptomatic relief. His temperature decreased to approximately 99°F. rectally after he had been placed on bed rest.

Course: An intensive study was undertaken in an attempt to make an etiologic and morphologic diagnosis of this patient's illness. A clinical diagnosis of acute diffuse interstitial pulmonary fibrosis was made. Meanwhile, the patient was treated with terramycin, streptomycin, penicillin, chloramphenicol, and erythromycin without any real symptomatic or clinical improvement. Six lymph nodes were excised from different sites, but microscopic examination revealed only moderate reactive hyperplasia and inflammation. A sternal marrow biopsy was done along with culture and was completely normal. A biopsy was taken from the gastrocnemius muscle and this too was unrevealing. Numerous sputum specimens were examined for tubercle bacilli, both by smear and culture, and were negative. Sputum was examined for fungi by smear and culture and this also was negative. Multiple studies of the sputum by Papanicolaou's technique were negative for abnormal or atypical cells. Skin tests with tuberculin, coccidioidin, histoplasmin, and blastomycin were negative. A liver biopsy was done under local anesthesia and this was reported as negative.

During this period of investigation, the patient became slowly and progressively worse. His dyspnea increased and was associated with a moderate increase in cyanosis, even when receiving oxygen. After approximately one month of hospitalization, the patient appeared premoribund. He was markedly dyspneic and showed marked cyanosis of the face, lips, nail beds, and skin. Although two previous patients with diffuse pulmonary fibrosis had been reported to have received corticotropin without any improvement, it was decided to give this patient corticotropin as a last resort measure.

Corticotropin (ACTH) and cortisone: Accordingly, the patient was given corticotropin (ACTH) by slow intravenous drip, receiving twenty-five units during an eight-hour period. Because of the evidence of increasing cardiac insufficiency, he was also digitalized. After four days of corticotropin therapy, the patient began to show marked improvement. He was able to do without supplementary oxygen for short periods of time during the day, and the cyanosis had improved considerably. The corticotropin was continued and, by the twelfth day following the onset of therapy, the patient's relief was so marked that he was able to get out of bed for the first time during his hospital stay. Roentgenographic examination of the chest shortly afterward revealed evidence of almost complete clearing of the interstitial infiltrations previously noted (figure 2). After approximately three weeks of corticotropin therapy, the patient was able to walk about the ward at a leisurely rate without dyspnea.

There was no evidence of cyanosis. He began to gain weight and was symptomatically much improved.

At this time, the corticotropin was discontinued and the patient was given cortisone tablets orally, receiving 200 mg. the first day, and then the amount was decreased slowly so that he was maintained with 100 mg. per day. On this regimen his improvement continued. He was able to return to his home for a period of one week, during which time he continued to take his cortisone tablets. Repeated chest roentgenograms revealed that the previously described clearing was being maintained. Electrocardiograms done serially showed the development of definite evidence of myocardial disease with changes compatible with anterolateral ischemia. During this period of time the diagnosis was still in doubt, although it was the continued feeling of the staff who saw this patient that he represented and fulfilled all of the clinical criteria for a diagnosis of diffuse interstitial pulmonary fibrosis.

Withdrawal of cortisone: After approximately two months of cortisone therapy, it was decided to withdraw the steroids slowly and carefully watch the patient for further developments. This was done during a period of one and one-half weeks, during which time corticotropin was given in an attempt to stimulate any adrenal depression which might have resulted from the cortisone therapy. He continued to remain symptomatically well and showed no changes in the roentgenogram of the chest on the day following the total discontinuance of steroid therapy.

On the night prior to the patient's death, he was able to walk about the hospital without any difficulty and with no unusual complaints. Approximately four days following the discontinuance of steroid therapy, the patient awoke with marked dyspnea. Roentgenographic examination of the chest was made at once and this revealed a marked increase in interstitial infiltrations comparable in degree to the examination on admission (figure 3). Corticotropin was started immediately and the patient received 30 units by slow drip intravenously during an eight-hour period. In addition, within the next twelve hours, he was given 800 mg. of cortisone. During the next twenty-four hours, the patient's dyspnea and cyanosis progressively became more severe, and he quietly died. His death was one of acute asphyxiation.

Necropsy

The body was that of an asthenic, adult white male. The essential gross findings were as follows:

Thoracic cavity: The mediastinum was in the midline, and the lungs completely filled their respective chest cavities. The parietal pleura was clear and smooth and gray in appearance. The visceral pleura was everywhere smooth, pale gray, and showed linear streaks of anthracotic pigment. Approximately 25 cc. of a clear serous fluid were present in the pleural space on each side. In the anterior superior mediastinum just above the right innominate vein was a mass of discrete and matted lymph nodes, the largest one measuring approximately 2.5 by 0.5 cm. Nodes of this type were also quite prominent in the tracheobronchial region and were noted along the soft tissue anterior to the vertebral column in the posterior mediastinum. On cut section, these nodes showed a moderately firm, homogenous gray lymphoid type of tissue. In some other instances, the tissue was noted to be granular and varied in color from black to pink to gray. The nodes were scattered, discrete, or confluent with no consistent visible pattern. The trachea, esophagus, great vessels, and pericardium appeared normal. The lungs together weighed approximately 1,700 gm. All of the lobes of both lungs were quite firm and rubbery to palpation. There was a small area in the anterior edge of the right middle lobe which was slightly softer to palpation than the remainder of the lungs. In all of the lobes of both lungs, on cut section, the tissue appeared predominately gray with small flecks of black anthracotic pigment scattered here and there. Sections were firm, smooth, and somewhat glazed. The small bronchioles were quite distinct. The mucosa of the bronchi was gray, and no significant amount of secretions was noted. The pulmonary vessels were not remarkable. No solitary scars, gross abscesses, or any areas of caseation were seen (figures 1 and 5).

Pericardial cavity: The pericardium was smooth, pale gray, fibrous tissue and appeared

Case 1

FIG. 1. (Left) Roentgenogram of the chest shortly after admission.
FIG. 2. (Center) Roentgenogram of the chest following the institution of corticotropin therapy.
FIG. 3. (Right) Roentgenogram of the chest following acute relapse.

FIG. 4. Case 1. Post-mortem roentgenogram of the lungs showing almost complete absence of aerated lung.

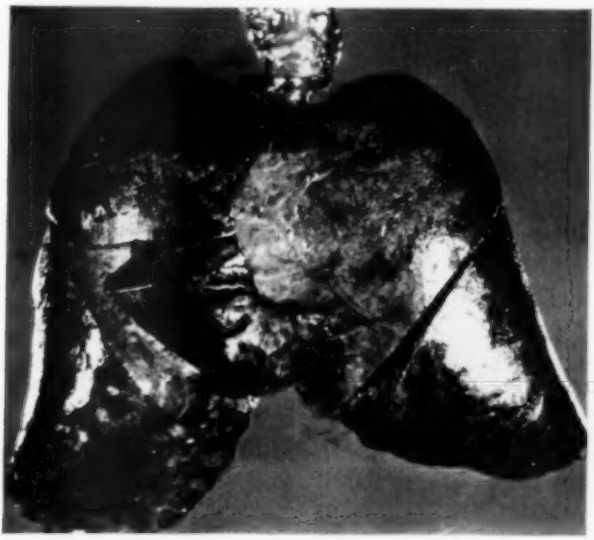

FIG. 5. Case 1. The lungs at autopsy showing a firm, rubbery consistency.

grossly normal. The cavity contained approximately 20 cc. of a clear serous fluid. The gross contour of the heart was normal; the total weight was 300 gm. The epicardium appeared clear. The endocardium was clear, although some post-mortem blood clots were adhered to the wall of the left ventricle. The valves appeared grossly normal and their measurements were within normal limits. The wall of the left ventricle measured 1.5 cm. and that of the right ventricle 0.5 cm. The chambers of the heart did not appear to be dilated. The myocardium was firm, tan, and homogenous everywhere. The coronary vessels were clear everywhere and patent. No thrombi were observed in the blood vessels leading to or from the heart or in the pulmonary vessels.

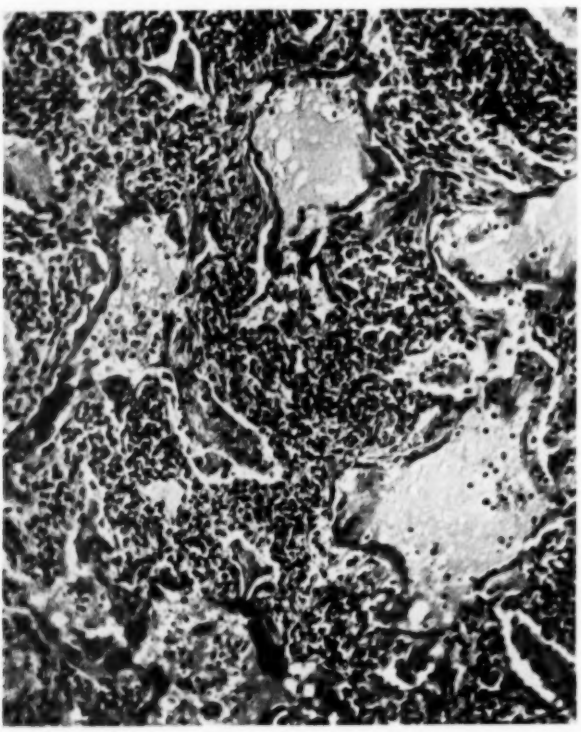

FIG. 6. Case 1. Photomicrograph of the lung. (× 100) Note the hyalinization of the alveolar membrane.

Abdominal cavity: The liver appeared grossly normal. The spleen appeared grossly enlarged, but not deformed. The total weight of the spleen was 400 gm. and its gross architecture on cut section was normal. The total weight of the liver was 1,840 gm. and the gross and cut section architecture was normal. The adrenal glands together weighed 12 gm. and on section the cortex and medulla appeared grossly normal. The mesentery of the small bowel contained numerous firm large lymph nodes, some appearing discrete and others matted together. On cut section, these showed a pale gray homogenous type of tissue.

Lymph nodes: Numerous enlarged lymph nodes, some of which measured as much as 2.5 cm. in the greatest diameter, were present along the entire vertebral column from approxi-

mately *T*-four to *L*-five. These nodes were both discrete and confluent. They also extended along the splenic artery to the hilum of the spleen and along the external iliac arteries to the inguinal ligament. The remainder of the gross necropsy was not unusual.

The provisional anatomic diagnosis at the time of necropsy was pulmonary fibrosis, etiology as yet undetermined.

Microscopic examination: Microscopic examination of sections taken from all lobes of the lungs showed a picture of a diffuse interstitial pneumonitis as well as numerous hyaline membranes lining the alveolar walls. There was a marked decrease in the number of functioning alveoli as well as a constriction to almost slitlike spaces of many of those remaining.

FIG. 7. Case 1. Photomicrograph of the lung showing the acute interstitial pneumonitis and early fibrosis. (× 100)

A moderate amount of interstitial fibrosis was noted in all of the lobes, although various sections showed different stages and degrees of fibrosis. Numerous dilated capillary and small blood vessels were seen surrounded by the interstitial fibrous tissue and fibroblasts. An occasional islet of large epithelioid type of cells was noted in several sections, but these did not assume the appearance of tubercles or sarcoid. Conspicuous hyaline membranes were obvious within the alveoli and lining the alveolar walls. Eosinophils were rare. There was an absence of cellular infiltrate within the alveoli or bronchioles. Very few polymorphonuclear leukocytes were seen in any of the sections, the main cellular infiltrate consisting of lymphocytes, occasional phagocytes bearing pigment, rare giant cells, and numerous fibro-

blasts. Some of the alveolar lining cells appeared to be hyperplastic (figures 6 and 7). Microscopic examination of the spleen and lymph nodes revealed only a picture of a reactive hyperplasia. The heart showed only slight hypertrophy of the individual muscle fibers with no other obvious abnormalities. Gram stains and acid-fast stains of various sections of the lungs confirmed the absence of stainable bacteria within the alveoli themselves. Cultures of the lungs and lymph nodes revealed only a nonhemolytic *Staphylococcus aureus*, which was thought to be a contaminant. Cultures for fungi and tubercle bacilli were negative. Portions of the lungs and lymph nodes were immediately frozen and later injected into egg embryos and mice in a search for virus organisms, particularly of the psittacosis group. At the time of publication, these virus studies have failed to reveal any significant findings, although this study still is in progress.

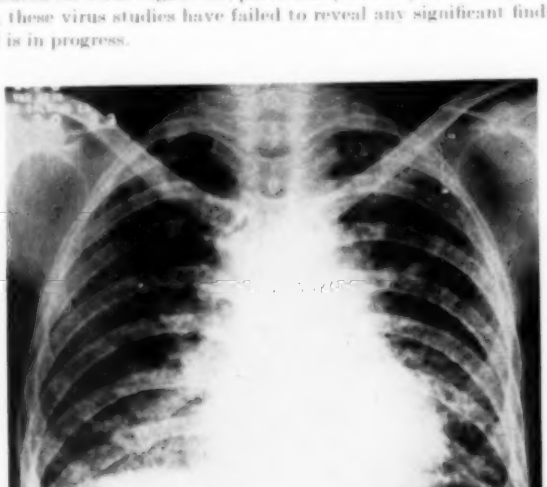

FIG. 8. Case 2. Roentgenogram of the chest showing the mottled dense interstitial infiltrate.

Case 2. J. W. S., a 38-year old clerical worker, was first seen in February, 1952, because of insidious slowly progressive dyspnea of approximately four years' duration. The only other complaint that the patient had was of moderate sputum production of approximately 15 to 30 cc. of a clear whitish sputum daily. At the time of onset, the patient noted dyspnea after moderate exertion. This progressed slowly so that approximately two years prior to admission the patient noted that he became dyspneic upon walking two or three blocks. At the time of admission, the patient gave a history of being comfortable while at rest or with mild exertion; but, if he attempted to walk rapidly or run for twenty-five yards, he became moderately dyspneic. Examination at that time revealed fine crepitant rales at both bases posteriorly toward the end of inspiration. There was mild distention of the neck and arm veins with an accentuation of the pulmonic second sound. Roentgenograms of the chest revealed a diffuse bilateral interstitial type of infiltration (figure 8). Skin tests with tuberculin, coccidioidin, and histoplasmin were negative. No specific diagnoses was made on that admission and the patient was subsequently discharged with no specific medication.

He was seen again in May, 1952, because of increasing dyspnea. At that time, there was a slight diminution of resonance to percussion over both lungs. The breath sounds were

slightly diminished and fine moist rales were heard at both lung bases, particularly at the end of inspiration. Multiple views of the chest on roentgenographic examination revealed again the generalized interstitial infiltration bilaterally with what appeared to be some progression since the previous examination. The electrocardiogram had also changed and showed evidence of right ventricular strain. At that time, an extensive effort was made to establish a diagnosis. A bilateral scalene node dissection was done with cultures of the nodes. Microscopic examination revealed only mild reactive hyperplasia and the cultures were negative for bacteria, fungi, or tubercle bacilli.

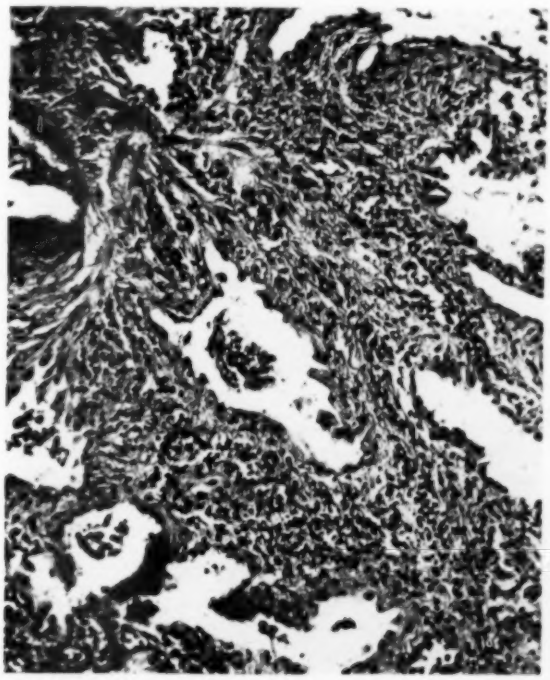

FIG. 9. Case 2. Photomicrograph of the lung showing severe interstitial fibrosis. (× 100)

Thoracotomy and biopsy: Because of the progressive nature of the patient's illness, an open thoracotomy was done and a biopsy of the left upper lobe of the lung was performed. At surgery, gross inspection of the lung revealed it to be covered and puckered with numerous scars. The surgeon described the lung as appearing similar to a cirrhotic type of liver. Microscopic examination of the lung revealed a picture of chronic interstitial pneumonitis of a mild degree with marked interstitial fibrosis. The cellular infiltrate consisted chiefly of lymphocytes. No eosinophils were seen. There was a marked increase in the amount of intersititial fibrous tissue and a mild increase in the number of fibroblasts. The number of alveoli was markedly decreased and an occasional hyaline membrane was seen within the alveolar cavity. Bronchioles appeared normal. No stainable bacteria were found within the lung or within the alveoli (figure 9). Additional portions of the lungs were finely ground under sterile precautions and were cultured and injected into guinea pigs. These studies were completely negative for any organism.

Corticotropin: The patient was again discharged, but he returned in January, 1953, with a history that his dyspnea had slowly progressed. At the time of his readmission, he was dyspneic at rest and during mild conversation. There was a tinge of cyanosis of the nail beds and lips. He was placed on bed rest and oxygen was given by tent. This gave the patient some symptomatic relief so that he was able to remain out of oxygen as long as he was completely quiet in bed. He was given a course of intravenous corticotropin during a thirty-day period, with no objective improvement in his dyspnea or chest roentgenographic findings. Subsequently, while still receiving corticotropin, his slow downhill course continued and he died in March, 1953. His death was one of chronic asphyxiation.

Necropsy

At autopsy, the lungs were slightly contracted but weighed 2,600 gm. No unusual secretions were noted within the tracheobronchial tree. On the surfaces of the lungs were numerous whitish scars, giving a hobnail appearance. These areas were symmetrically distributed over the entire lung area and were of approximately equal size. The lungs were firm, slightly rubbery in consistency, and cut with a slightly gritty sensation. The microscopic findings were as previously described on the biopsied specimen. Since the pathologic examination of the tissues removed are consistent with a diagnosis of diffuse interstitial pulmonary fibrosis as originally described by Hamman and Rich, it is believed that this case is of the longest duration reported.

COMMENT

It may be that the case reports of the syndrome of diffuse interstitial fibrosis of the lungs which are accumulating in the literature represent end results of different clinical syndromes. It is conceivable that our present state of knowledge about this disease is comparable to the state of knowledge about the kidney when the term "cirrhosis of the kidney" was popular. As more knowledge and evidence about this disease accumulates, a more specific etiologic and clinical explanation might be made than is implied by the generally nonspecific term of diffuse interstitial fibrosis. As has been shown by the various case reports, the duration of this disease can vary from four weeks to four years. It can be rapidly and progressively fatal or it can have an insidious, slowly progressive course. The two case reports herein cited conform to the pathologic criteria as originally described by Hamman and Rich. The difference between the two cases is in the apparent stage of the disease in which the examination was made. The absence of eosinophils in the first case could possibly have been due to the steroid therapy which the patient received.

The first patient herein cited is the third reported case in which the extensive use of corticotropin and/or cortisone was made. As was seen, the use of corticotropin in the patient who was almost moribund resulted in a complete symptomatic regression with marked and rapid improvement in dyspnea and cyanosis. This symptomatic improvement was correlated with roentgenographic clearing of the finely interstitial infiltrations which were previously present. The fact that the roentgenographic abnormalities cleared so rapidly on corticotropin therapy indicates that at least part of the infiltration seen was not fibrosis but represented a reversible process. This was later confirmed by pathologic examination in which all stages of interstitial pneumonitis and fibrosis were seen. Upon cessation of cortisone and corticotropin therapy, the patient had a sudden and much exaggerated relapse which was refractory to further therapy. This relapse was again correlated with roentgenographic findings of marked interstitial infiltration.

The fact that at pathologic examination a moderate amount of interstitial fibrosis was already present indicates that part of the disease had already run its course prior to the corticotropin therapy or else that the fibrosis is insidious and progressive despite the clinical regression caused by corticotropin therapy. The theory that the main pathologic

features of this disease represent tissue reaction indicates that the further use of corticotropin and cortisone therapy in additional acute cases might be of great value. Such use is strongly encouraged with one additional warning. Once the patient is placed on corticotropin or cortisone therapy, he should be continued on this therapy indefinitely. Discontinuance of therapy in the first patient of this report apparently resulted in a sudden and much exaggerated relapse, leading to sudden death.

The second case is of interest in that it appears to represent the longest duration of this syndrome yet recorded.

SUMMARY

Two cases of Hamman-Rich syndrome of diffuse interstitial pulmonary fibrosis are reported. The patients differed in their duration of symptoms and in the stage at which pathologic examination was made.

The use of corticotropin or cortisone therapy may be life-saving in patients with acute rapidly progressing disease.

Once begun, steroid therapy probably should be continued indefinitely, lest an acute and exaggerated relapse occur.

The etiology of this disease is as yet undetermined. Portions of the lungs and lymph nodes which were injected into mice and egg embryos in a search for viruses have failed to reveal any significant results.

SUMARIO

Fibrosis Intersticial Difusa de los Pulmones

Preséntanse 2 casos del síndrome de Hamman-Rich de fibrosis pulmonar intersticial difusa. Los enfermos discrepaban en la duración de sus síntomas y en el período en que se les hizo el examen histopatológico.

El empleo de la terapéutica con corticotropina o cortisona puede salvar la vida a los enfermos que tienen la dolencia en su forma aguda y de rápida progresión.

Una vez iniciado el tratamiento con esteroides, con toda probabilidad tiene que ser continuado indefinidamente, para que no sobrevengan recidivas agudas y exageradas.

La etiología de la afección está aun por determinar. Los ratones y embriones de huevo en los que se inyectaron porciones de los pulmones y ganglios linfáticos en busca de virus no revelaron resultados de importancia.

RESUME

Fibrose interstitielle diffuse du poumon

Les observations de cas de syndrome de Hamman-Rich, fibrose interstitielle pulmonaire diffuse, sont rapportées. Chez ces malades, il existait des différences quant à la durée des symptômes et le stade auquel les examens ont été pratiqués.

La thérapie par la corticotrophine ou la cortisone peut être salvatrice quand l'affection est aiguë et progresse rapidement.

Lorsque cette thérapie est instaurée, elle devra probablement être poursuivie indéfiniment pour éviter l'apparition de rechutes aiguës et sévères.

L'étiologie de cette affection est encore imprécise. L'inoculation à la souris et à l'embryon de poulet de fragments de poumons et de ganglions lymphatiques pour la recherche de virus n'a pas donné de résultats significatifs.

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REFERENCES

(1) Hamman, L., and Rich, A. R.: Acute diffuse fibrosis of the lungs, Bull. Johns Hopkins Hosp., 1944, *74*, 177.
(2) Beams, A. J., and Harmon, O.: Diffuse progressive interstitial fibrosis of the lungs, Am. J. Med., 1949, *7*, 425.
(3) Cox, T. R., and Kohl, J. M.: Diffuse fibrosing interstitial pneumonitis (interstitial fibrosis of the lungs), Am. J. Clin. Path., 1952, *22*, 770.
(4) Eder, H., Van Zandt Haron, C., and Thorn, G.: Report of a case of acute interstitial fibrosis of the lungs, Bull. Johns Hopkins Hosp., 1945, *76*, 163.
(5) Katz, H. L., and Auerbach, O.: Diffuse interstitial fibrosis of the lungs, Dis. of Chest, 1951, *20*, 306.
(6) Heppleston, A. G.: Chronic diffuse interstitial fibrosis of the lungs, Thorax, 1951, *6*, 426.
(7) Golden, A., and Tullis, J. F., Jr.: Diffuse interstitial fibrosis of the lungs, Mil. Surgeon, 1949, *105*, 130.
(8) Mallory, T. B.: Pathology of pulmonary fibrosis, Radiology, 1948, *51*, 168.
(9) Rubin, E. H., Hahn, B. S., and Pecker, D.: Diffuse interstitial fibrosis of the lungs, Arch. Int. Med., 1952, *36*, 827.
(10) Callahan, W. P., Jr., Sutherland, J. C., Fulton, J. K., and Kline, J. R.: Acute diffuse interstitial fibrosis of the lungs, Arch. Int. Med., 1952, *90*, 168.
(11) MacMillan, J. M.: Familial pulmonary fibrosis, Dis. of Chest, 1951, *20*, 426.
(12) Editorial: Acute diffuse interstitial fibrosis of the lungs, J.A.M.A., 1953, *151*, 215.
(13) Potter, B. P., and Gerber, I. E.: Acute diffuse interstitial fibrosis of the lungs: A case report, Arch. Int. Med., 1948, *82*, 113.
(14) Peabody, J. W., Peabody, J. W., Jr., Hayes, E. W., and Hayes, E. W., Jr.: Idiopathic pulmonary fibrosis; its occurrence in identical twin sisters, Dis. of Chest, 1950, *28*, 330.
(15) Peabody, H. D., Moersch, J. H., and Edwards, J. E.: Clinically indeterminate pulmonary fibrosis: A pathologic study, Proc. Staff Meet., Mayo Clin., 1951, *26*, 266.
(16) Auerbach, S. H., Mims, O. M., and Goodpasture, E. W.: Pulmonary fibrosis secondary to pneumonia, Am. J. Path., 1952, *28*, 69.
(17) Tumulty, P. A., Berthrong, M., and Harvey, A. M.: A peculiar pneumonia associated with retinal cytoid bodies, Bull. Johns Hopkins Hosp., 1951, *88*, 239.

COEXISTENT PULMONARY TUBERCULOSIS AND SYSTEMIC BLASTOMYCOSIS[1]

Report of a Case Successfully Treated with Streptomycin, Para-Aminosalicylic Acid, and Stilbamidine

THOMAS C. BLACK AND HENRY M. WILSON

(Received for publication April 30, 1953)

INTRODUCTION

The coexistence of tuberculosis and blastomycosis is rare. The present writers were able to find only two cases of this combination (1) in a review of the literature. Accordingly, they are reporting a third case of such a double infection.

North American blastomycosis is caused by a fungus, *Blastomyces dermatitidis*. Blastomycosis may be either a simple cutaneous infection with a good prognosis or a systemic infection with a grave prognosis. The mortality rate of the systemic type is approximately 85 per cent (2, 3). The diagnosis is made by isolation of *B. dermatitidis* in cultures and its characteristic appearance in tissue specimens (4). In a review of the literature in 1939, Martin and Smith (2) accepted only 23 per cent of the 347 cases reported as blastomycosis.

Treatment of blastomycosis has been far less satisfactory than that of tuberculosis. Numerous authors have found the sulfonamides, iodides, penicillin, Promin, and streptomycin ineffective in blastomycosis. Most agents which are fungicidal *in vitro* and in animals have been found too toxic for clinical use. Schoenbach and Greenspan, in a comprehensive review of the aromatic diamidines (5), suggested the use of stilbamidine in the treatment of blastomycosis. The subsequent preliminary report of its use by Schoenbach and co-workers (6) led us to employ it in the following case.

CASE REPORT

A white male, Louisiana farmer, aged 33, was admitted to the Medical Service, January 2, 1950, for treatment of cramping pain in the epigastrium and a 10-pound weight loss during the preceding eighteen months. He had been discharged from the army in 1945 after five years of service which included residence in Belgium, France, Germany, and Holland. He had been treated for infectious hepatitis and syphilis in 1942. He had had herpes zoster in 1947. He smoked a package of cigarettes daily. His parents were living, and his father had "a stomach ulcer." There was no family history of tuberculosis.

Physical examination showed a poorly nourished white man who appeared chronically ill. The oral temperature was 100.2° F. The teeth were carious; dental hygiene was poor. Bronchial breathing and fine crepitant post-cough rales were heard in the upper third of the right chest posteriorly. There was soreness in the epigastrium on deep palpation. No masses were felt in the abdomen. Small discrete lymph nodes were palpated in both inguinal regions.

The initial blood count showed: 10,850 leukocytes per cu. mm. with 66 per cent mature neutrophils, 28 per cent lymphocytes, 4 per cent monocytes, and 2 per cent eosinophils; 4.8 million erythrocytes per cu. mm.; 11 gm. hemoglobin; and erythrocyte sedimentation rate of 20 mm. per hour (Cutler method). The urinalysis, gastric analysis, and three feces examinations were normal. A serologic test for syphilis (Kahn) was negative.

Diagnosis of pulmonary tuberculosis: A roentgenogram of the chest (figure 1) showed infiltrative changes extending from the right subclavicular region to the right interlobar

[1] From the Tuberculosis and Medical Services, Veterans Administration Hospital, Alexandria, Louisiana.

fissure. A 2 cm. cavity was present. Examination of a sputum smear showed acid-fast bacilli, which resembled *M. tuberculosis*. The tuberculin test was 2-plus with first-strength PPD. The patient was transferred to the Tuberculosis Service on January 5.

Further questioning revealed that the patient had been in good health until the spring of 1948, when he noticed he was tiring easily. He also gradually began to have a slight fever and to cough and raise a small amount of sputum daily. He had occasional intestinal upsets consisting of epigastric cramping pain, gas, and vomiting which occurred one to two hours after eating. The pain was not relieved by food or soda. The patient was treated for malaria without relief. During 1949, the intestinal upsets became more frequent, approximately every month. The tiredness and weakness increased. The amount of sputum gradually increased until on admission he was raising 15 cc. of yellowish white sputum daily.

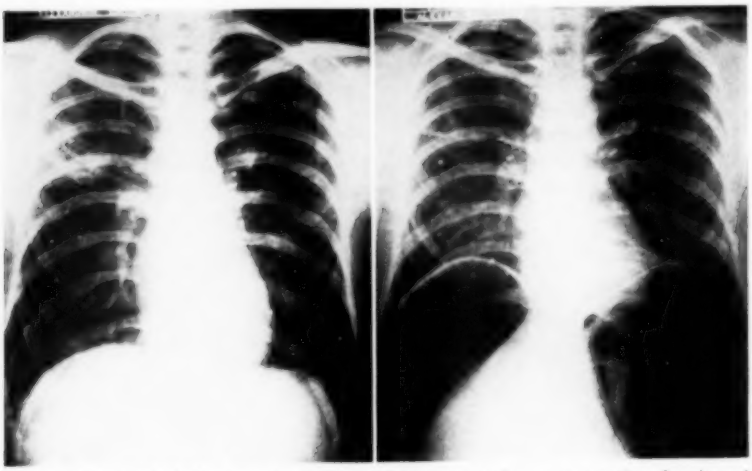

FIG. 1. (Left) Admission roentgenogram of the chest, January 3, 1950, showing right upper lobe infiltration with cavitation.

FIG. 2. (Right) Roentgenogram of the chest, November 13, 1950, showing only residual fibrosis following rest, chemotherapy, and pneumoperitoneum.

There had been no hemoptysis. The patient continued to work until admitted to the hospital. He believed his illness was due to "indigestion." Subsequent roentgenographic studies of the gastrointestinal tract were normal. The gastrointestinal symptoms disappeared (after treatment with belladonna and phenobarbital).

When two other sputum specimens were positive on smear for acid-fast bacilli, pulmonary tuberculosis, moderately advanced, active, was diagnosed. Treatment consisted of bed rest, to which right pneumothorax was added on January 18, 1950. Because of partial atelectasis of the right upper lobe, the pneumothorax was discontinued on January 26, 1950; re-expansion occurred. The next day the patient complained of sore throat, and 50,000 units of penicillin were administered every three hours for eight days and again for seven days beginning on February 15, 1950. Penicillin had no effect on the pulmonary disease and afforded only slight relief to the throat. Bronchoscopic examination on February 13, 1950, revealed mucoid secretion coming from the right upper lobe but no evidence of endobronchial tuberculosis.

Antituberculous therapy: Chemotherapy consisting of 1 gm. of streptomycin daily and 12 gm. of para-aminosalicylic acid (PAS) daily was given from February 20 to June 20. Pneumoperitoneum was instituted February 21, 1950.

A total of eight different sputum specimens were positive on smear for acid-fast bacilli.

The erythrocyte sedimentation rate, which had been 20 mm. in one hour on admission, became 12 mm. on June 7, and 3 mm. on December 8. The leukocyte count, which had been 10,850 per cu. mm. on admission, was 8,400 on June 7. The neutrophils decreased from 66 per cent to 60 per cent in the same period.

The combination of rest, chemotherapy, and pneumoperitoneum produced cavity closure, sputum "conversion," diminution in symptoms, and gradual improvement in the patient's general condition. A right phrenemphraxis was performed on November 21. Pneumoperitoneum refills had caused complaints of abdominal pain by the patient, and these were discontinued on November 24. Roentgenograms of the chest had shown gradual resolution of the infiltration and, in November, only some residual fibrosis remained (figure 2). Sputum cultures obtained in January, 1951, were negative for *M. tuberculosis* and fungi. The patient was asymptomatic, and graduated exercise was begun.

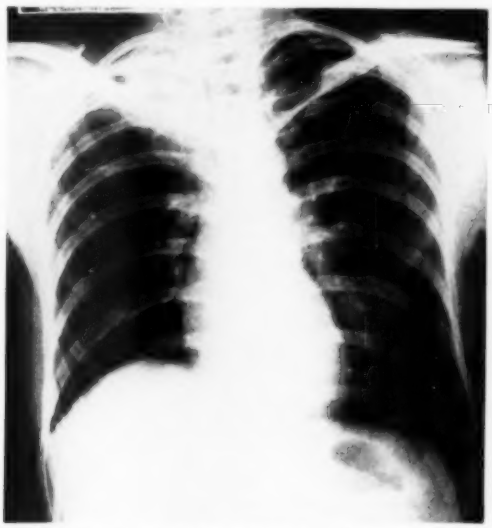

FIG. 3. A new infiltration was noted as shown on the roentgenogram taken May 28, 1951, and showed no change with streptomycin and PAS therapy. Lesion prior to stilbamidine therapy.

Appearance of blastomycosis: In April, he began to cough, raise sputum, and have a slight elevation of temperature. Blood counts at this time showed: 15,900 leukocytes per cu. mm. with 77 per cent neutrophils, 18 per cent lymphocytes, 2 per cent monocytes, 1 per cent eosinophils, and 2 per cent basophils; 4.5 million erythrocytes per cu. mm.; 12.8 gm. hemoglobin; and an erythrocyte sedimentation rate of 22 mm. per hour. Ten days later the leukocyte count had increased to 18,200 per cu. mm. and the sedimentation rate to 28 mm. A roentgenogram showed a new homogenous infiltration in the right upper lobe (figure 3). Several sputum smears were negative for acid-fast bacilli. Although cultures were not yet available, the chest lesion was assumed to represent a reactivation of tuberculosis. On April 18, treatment with 1 gm. of streptomycin and 12 gm. of PAS daily was resumed. Cultures and additional smears from the sputum were all subsequently negative for *M. tuberculosis*.

On approximately May 25, 1951, the patient complained of pain in the left elbow joint.

This persisted. Roentgenographic examination of the joint three days later revealed no abnormality. The joint gradually became swollen and the overlying skin became hot and red. In June, scattered ulcers appeared on the skin. Another roentgenogram of the elbow in early July showed some bony rarefaction and destruction of the articular surfaces. By mid-July ulcers were present on the left pretibial region, right Achilles area, the interscapular region, and over the right deltoid. The skin lesions began as subcutaneous nodules which suppurated, forming abscesses which spontaneously ruptured and formed crusted lesions which failed to heal (figure 4).

A fluctuant subcutaneous abscess on the anterior aspect of the right thigh had gradually developed in one month and, on July 25, glary mucoid pus was aspirated. This material showed the tissue phase of *Blastomyces dermatitidis* on direct microscopic examination.

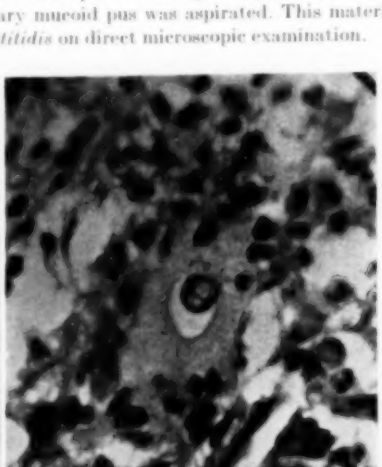

FIG. 4. (Left) Photograph of right wrist with typical crusted cutaneous lesion of blastomycosis.

FIG. 5. (Right) Photomicrograph of biopsy specimen of wall of a skin lesion revealing granulomata in the dermis and the typical yeastlike organisms of *B. dermatitidis*.

Within six days, *B. dermatitidis* was identified in cultures[2] from both sites. Three subsequent cultures of pus from the elbow joint were positive. Cultures of the same material failed to show *M. tuberculosis* after sixty days of incubation. Two sputum cultures showed *B. dermatitidis*. A biopsy specimen of the wall of a skin lesion (figure 5) showed several minute granulomata in the dermis and scattered yeastlike organisms. An intradermal test with blastomycin was positive. Streptomycin-PAS therapy was discontinued. Chest films showed no change.

Stilbamidine therapy: Stilbamidine[3] was first administered to the patient on October 2, 1951, according to the empiric schedule reported by Schoenbach (7). The patient was given

[2] The fungus was grown on Thompson's medium, which consists of 5 per cent blood agar with 1 per cent dextrose, to which 25 units of penicillin and 25 γ of streptomycin per. ml. of medium had been added.

[3] Stilbamidine (4,4 stilbenedicarboxamidine) was furnished by Merck & Co., Inc., Rahway, New Jersey.

the drug dissolved in 250 cc. of saline intravenously so slowly that the infusion took one hour. Exposure of the solution to light was avoided. The patient received three courses of stilbamidine, each consisting of 50 mg. initially, none the second day, 100 mg. the third day, followed by 150 mg. every other day for fifteen treatments. A ten-day rest period was allowed between courses. He received a total of 6.75 gm. of the drug.

After three weeks of stilbamidine treatment, the skin lesions had improved; there was less inflammation about the elbow and less drainage from it. Healing took place slowly. The cough and sputum disappeared. The leukocyte count on November 23, 1951, showed 8,150 leukocytes per cu. mm., of which 45 per cent were neutrophils, 50 per cent lymphocytes, 1 per cent monocytes, and 4 per cent eosinophils. The erythrocyte sedimentation rate was 5 mm. per hour.

FIG. 6. (Left) Roentgenogram of the chest, December 5, 1951, while undergoing stilbamidine therapy, showing clearing of the pulmonary lesion and only residual fibrosis.

FIG. 7. (Right) Roentgenogram of the chest, January 19, 1953, on follow-up examination after completion of therapy and discharge showing no change in comparison with the roentgenogram of December 5, 1951. When the patient was again seen on July 1, 1953, for follow-up examination, he was in good health and chest roentgenograms showed no change when compared with the film of January 19, 1953.

The elbow joint healed with partial ankylosis, and roentgenograms showed osteoporosis. All of the skin lesions healed completely. Nine different sputum cultures were sterile after treatment. Blood counts in March, May, and August, 1952, were normal and the erythrocyte sedimentation rates were 4 to 6 mm. per hour.

A chest roentgenogram in December, 1951 (figure 6) showed only residual fibrosis.

The patient showed progressive improvement and he was given graduated exercise. When he was discharged on October 6, 1952, the tuberculosis was arrested and the blastomycosis was cured.

A roentgenogram of the chest in January, 1953, (figure 7) showed essentially no change from December, 1951. When the patient was last seen on March 27, 1953, he was in good health. There was no evidence of facial neuropathy resulting from stilbamidine. The chest films continued to show no change.

DISCUSSION

A young white man was found to have systemic North American blastomycosis while convalescing from pulmonary tuberculosis.

The diagnosis of tuberculosis was based on: history and symptomatology; roentgenograms of the chest showing lesions compatible with tuberculosis, including a cavity 2 cm. in diameter; acid-fast bacilli which morphologically resembled *M. tuberculosis* in smears of eight separate sputum specimens; and clearing of the chest lesions, "conversion" of the sputum, and clinical improvement following streptomycin-PAS treatment. Published reports (3, 8, 9) stress the ineffectiveness of streptomycin against *Blastomyces*. Hence, the improvement noted with chemotherapy indicates that blastomycosis did not play a major role in the early course of this man's illness.

The numerous smears positive for acid-fast bacilli during the acute phase of tuberculosis were accepted without confirmation by cultures. These smears should have been confirmed. The first sputum cultures were not made until January, 1951, and were negative for *M. tuberculosis* and pathogenic fungi.

Systemic North American blastomycosis was proved by the identification of *Blastomyces dermatitidis* in cultures from sputum, skin lesions, and drainage of pus from the elbow joint. These organisms were also demonstrated in a biopsy specimen from the wall of one of the skin lesions.

The pharmacology and mode of action of stilbamidine and related diamidines have been thoroughly reviewed by Schoenbach and Greenspan (5). It has been used for treatment of diseases other than blastomycosis.

Stilbamidine is a very toxic agent if it is exposed to light after being put up in solution. With precaution against exposure to sunlight, the only chronic toxic effect has been confined to a unique neuropathy consisting of paresthesia, anesthesia, hypalgesia, and numbness, usually confined to the face (10). The patient experienced these changes in his face to a mild degree. This was a calculated risk which was assumed considering the high mortality rate in systemic blastomycosis even without coexistent tuberculosis. The patient agreed with this decision and was not troubled by the neuropathy, which disappeared with the passage of several months' time. The mechanism of the neuropathy remains obscure.

SUMMARY

The coexistence of pulmonary tuberculosis and systemic North American blastomycosis is rare. One such case was successfully treated with streptomycin-PAS and stilbamidine. The patient remains well sixteen months after treatment.

SUMARIO

Coexistencia de Tuberculosis Pulmonar y Blastomicosis Generalizada

La coexistencia de la tuberculosis pulmonar y la blastomicosis norteamericana generalizada es rara. Un caso de ese género fué tratado con éxito con estreptomicina ácido para-aminosalicílico y estilbamidina. El enfermo continúa bien a los dieciséis meses del tratamiento.

RESUME

Tuberculose pulmonaire avec association de blastomycose généralisée

La coexistence de la tuberculose pulmonaire et de la blastomycose généralisée (type Amérique du Nord) est rare. Le traitement d'un de ces cas par la streptomycine associée à l'acide para-aminosalicylique et par la stilbamidine s'est montré efficace. L'état du malade demeure satisfaisant seize mois après le traitement.

Acknowledgments

The authors are indebted to Dr. G. M. Carrera, Associate Professor in Pathology at Tulane Medical School, New Orleans, Louisiana, for reviewing the section of a skin lesion; and to Mr. Frank Clifton, of our laboratory, for the cultures.

REFERENCES

(1) Feld, D. D., and Cadden, A. V.: Systemic blastomycosis, Dis. of Chest, 1949, *16*, 473.
(2) Martin, D. S., and Smith, D. T.: Blastomycosis (American blastomycosis, Gilchrist's disease): I. A review of the literature, Am. Rev. Tuberc., 1939, *39*, 275.
(3) Lowry, C. C., Kraeft, N. D., and Hughes, F. A., Jr.: Blastomycosis of the lung, Am. J. Surg., 1951, *81*, 676.
(4) Baker, R. D.: Tissue reaction in human blastomycosis, Am. J. Path., 1942, *18*, 479.
(5) Schoenbach, E. B., and Greenspan, E. M.: The pharmacology, mode of action and therapeutic potentialities of stilbamidine, pentamidine, propamidine, and other aromatic diamidines: A review, Medicine, 1948, *27*, 327.
(6) Schoenbach, E. B., Miller, J. M., Ginsberg, M., and Long, P. H.: Systemic blastomycosis treated with stilbamidine: A preliminary report, J.A.M.A., 1951, *146*, 1317.
(7) Schoenbach, E. B.: Personal communication to the authors.
(8) Whitaker, H. W., Jr.: North American blastomycosis, Arch. Path., 1949, *48*, 212.
(9) Littman, M. L., Wicker, E. H., and Warren, A. S.: Systemic North American blastomycosis, Am. J. Path., 1948, *24*, 339.
(10) Schoenbach, E. B., Miller, J. M., and Long, P. H.: The treatment of systemic blastomycosis with stilbamidine, Ann. Int. Med., 1952, *37*, 31.

Notes

THE EFFECT OF PARA-AMINOSALICYLIC ACID ON SILICATE RESTORATIONS (FILLINGS) OF TEETH

For some time the writer has observed that silicate fillings which had been placed in the teeth of sanatorium patients who were taking para-aminosalicylic acid (PAS) orally had disintegrated at a very rapid rate, and the matter seemed worthy of record. The PAS used was prepared by adding to 27.6 gm. of sodium para-aminosalicylic acid a sufficient quantity of water to make 100 cc. of solution. To this, a few drops of peppermint was added as a flavoring agent.

Dental silicate cements are the most widely used filling material in the front of the mouth. They are the combination of a liquid (phosphoric acid) and a powder (silica). Silicate powders are made by melting together silica, alumina, and fluxes and heating them until fusion is complete. The glass that results is crushed and ground to a fine powder. The liquid is a solution of phosphoric acid, partially neutralized with alumina and zinc oxide.[1]

All silicate cements are soluble and disintegrate in the mouth fluid to some extent. This decomposition begins during the first week and becomes less each week. Even though this filling material is considered temporary, if it is manipulated and placed properly in the cavity, one can expect a relatively long period of usefulness.

The above clinical observation precipitated the following experimental procedure:

The S. S. white silicate was selected because it met American Dental Association specification No. 9, which requires:

> *Time of setting:* The time shall not be less than three or more than eight minutes.
> *Ultimate compressive strength:* The ultimate compressive strength shall be more than 1,260 kg. per sq. cm. (approximately 18,000 pounds per square inch) for specimens crushed seven days after mixing.
> *Solubility and disintegration:* The solubility and disintegration of the cement shall be less than 2.0 per cent by weight when immersed for seven days in distilled water at 37°C. (99°F.).[2]

Four glass jars arranged as follows were prepared to receive the test specimen (table 1):

Jar Number

1 contained distilled water.
2 contained a sample of saliva obtained from a nontuberculous person not taking PAS.
3 contained a sample of saliva obtained from a patient taking liquid PAS. The samples were obtained four hours after the administration of the drug.
4 contained the liquid form of PAS.

The test specimens of silicate were prepared by holding the silicate in Cellophane strips between the fingers for a period of eight minutes after the slab had been cooled and

[1] SKINNER, E. W.: The Science of Dental Materials, W. B. Saunders Company, Philadelphia, 1946, p. 149.

[2] SKINNER, E. W.: The Science of Dental Materials, W. B. Saunders Company, Philadelphia, 1946, p. 390.

the silicate manipulated in the proper manner. Eight minutes were allowed in order that the silicate might have its maximum setting time.

The Cellophane strips were then removed and the disks were covered with cocoa butter. The disks were divided into approximately equal halves and placed into the four jars.

As may be seen in table 1, at twelve hours the test specimens in jars 1 and 2 showed no visible change. In contrast, the silicate specimens in jar 3 showed a small amount of disintegration, and the specimen in jar 4 demonstrated very definite disintegration. The

TABLE 1

RESULTS OF VARIOUS SOLVENTS, INCLUDING PARA-AMINOSALICYLIC ACID, ON SILICATE FILLING

(This Series of Experiments Contained Fifty Fillings per Series)

SPECIMENS CONTAINED IN GLASS JARS CLASSIFIED BY SERIES NUMBER AND CONTENTS	VISUAL OBSERVATIONS ON FIRST EXPERIMENT				AVERAGE AMOUNT OF WEIGHT LOST OR GAINED ON SECOND EXPERIMENT
	12 hours	24 hours	7 days	3 weeks	
I Distilled water and silicate filling	No visible change	No change	No change	No change	+2.4 mg.
IV PAS liquid and silicate fillings	Disintegration evident, with breaking of silicate edges and discoloration	Greater disintegration than previously with indications of the granulation of filling	Completely granular state	Completely dissolved	−45.57 mg.
II Saliva from nontuberculous persons with silicate fillings	No visible change	No change	No change	No change	+16.0 mg.
III Saliva from tuberculous patients taking PAS and having silicate fillings	Some visible disintegration	Some visible disintegration with discoloration	Very slow disintegration	Minor disintegration	−0.1 mg.

edge of the silicate had begun to break and the silicate disk had changed to a deep brown color.

At twenty-four hours (table 1), the results in jars 1, 2, and 3 were essentially the same as at twelve hours. In jar 4, disintegration was more evident visually. The silicate disk had begun to change from a solid to a granular state.

At one week (table 1) after the start of the experiment, the specimens not exposed to PAS (jars 1 and 2) showed no change. In jar 3, containing PAS, there was visual evidence that the silicate was being very slowly disintegrated. In jar 4 (containing PAS), the silicate had visually changed from a solid to a granular state and dissolution of the preparation was quite evident.

The results after three weeks of the experiment were the same as at the earlier observation periods except in jar 4. In this jar (containing PAS), the silicate disk had completely disintegrated and dissolved. Indeed, there was no visible evidence that the silicate disk had ever been placed in the para-aminosalicylic acid solution.

The physical change noted in the structure of the silicate disk in the fourth container (containing PAS) of the first experiment was so evident that a second series of experiments was conducted, using a larger number of specimens with a weekly recording of weights. In this subsequent series, the change from the solid to a granular state did not entirely parallel the change noted in the first series, but the change in the second series was gradual, making it possible to observe the surface structure of the silicate from week to week.

After one week of exposure to PAS in water or saliva, a change could be noted in the surface of the silicate in the form of cracking. This cracking was very pronounced after a two-week period and, at the end of the second week, the surface was completely covered with cracks which formed a weblike configuration. At this stage, the slightest pressure caused the silicate to break.

This observation alone would lead one to question the practicability of the continued use of silicates as restorative material in those cases in which it would be subjected to para-aminosalicylic acid. The rapid cracking of the surface of the filling material should lead to a disinclination of the dentist to seek silicates as restorative material in these cases.

In summary: It has been observed repeatedly that patients taking para-aminosalicylic acid (PAS) orally experience rapid deterioration of the silicate fillings commonly used in repair of the teeth. In an experimental study of this phenomenon, it has been observed that very little visible change is noted when the silicate is subjected to distilled water and saliva from patients not receiving PAS. However, a questionable change is noted when silicates are submerged in saliva from a tuberculous patient who is taking PAS. The solubility and disintegration of silicate cement when exposed to PAS is greater than 2 per cent by weight when immersed for seven days. The crushing strength is greatly reduced. Constant exposure of silicate to PAS results in disintegration with final dissolution and a restoration which is irreparably ruined. Therefore, silicate cements should not be indicated as restorative material in those cases in which it is necessary to administer para-aminosalicylic acid orally.

Acknowledgment

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March 26, 1953

THE USE OF NEOTETRAZOLIUM CHLORIDE IN CULTURES OF TUBERCLE BACILLI

In the clinical isolation, identification, or drug-susceptibility testing of the tubercle bacillus, one of the primary difficulties is the time required before reporting the presence or absence of the organisms or their drug susceptibility or resistance.

In studies on the cytology of the tubercle bacillus and other organisms, the tetrazolium salts have been found to be exceedingly useful for cytochemical studies concerning the localization of mitochondria and, probably more specifically, bacterial flavins. One such bacterial enzyme, a reduced-DPN oxidase, has been demonstrated by Brodie and Gots[1] to reduce these salts *in vitro*. During the cytologic investigations in this laboratory,[2, 3] it was observed that only viable organisms would reduce the tetrazolium salts. Vandiviere, Gentry, and Willis[4] have presented a rapid viability testing procedure for tubercle bacilli, using triphenyltetrazolium chloride (TTC). Using carefully standardized suspensions, they show graphically that there is an excellent correlation between the amount of TTC reduced and the number of viable organisms present in a suspension. Shaffer, Kucera, and Spink[5], however, found that it was not possible to distinguish with neotetrazolium chloride a viable from a nonviable *Brucella* organism which had been phagocytosed by a leukocyte.

The present writers found that at certain concentrations the viable colonies would not only reduce the tetrazolium salt but would also continue to grow without apparent inhibition. These growing colonies were brightly colored and, hence, readily visualized. This suggested that it might be possible to incorporate one of the tetrazolium salts into medium upon which tubercle bacilli were cultured for identification and/or drug-susceptibility testing, and thereby visualize growth at an earlier date.

The data herein presented indicate that the incorporation of neotetrazolium in slants used for the primary isolation of tubercle bacilli presents substantial practical advantages. In testing for susceptibility to streptomycin, however, the presence of neotetrazolium may alter the apparent susceptibility to streptomycin of some organisms and, hence, give misleading results.

Jensen's modification of Löwenstein's egg medium was prepared according to the specifications of the Clinical and Laboratory Subcommittees of the American Trudeau Society for streptomycin-sensitivity determinations.[6] This medium was divided into equal parts from which were prepared sets of eight tubes containing the following concentrations of streptomycin and neotetrazolium chloride (NTC).[7]

[1] Brodie, A. F., and Gots, J. S.: Science, 1952, *116*, 588.

[2] Mudd, S., Winterscheid, L. C., DeLamater, E. D., and Henderson, H. J.: J. Bact., 1951, *62*, 459.

[3] Winterscheid, L. C., and Mudd, S.: Am. Rev. Tubere., 1953, *67*, 59.

[4] Vandiviere, H. M., Gentry, W. H., and Willis, H. S.: Am. Rev. Tubere., 1952, *66*, 95.

[5] Shaffer, J. M., Kucera, C. J., and Spink, W. W.: J. Exper. Med., 1953, *97*, 77.

[6] American Trudeau Society: Report of the Clinical and Laboratory Subcommittees, Am. Rev. Tubere., 1952, *65*, 105.

[7] Neotetrazolium chloride, Montclair Research Corporation, 4 Cherry Street, Montclair, New Jersey. The culture medium was prepared and the cultures were obtained in the Bureau of Laboratories, Pennsylvania Department of Health, and the experiments were conducted in those laboratories.

Tube No.	1	2	3	4	5	6	7	8
Neotrazolium chloride concentration, γ per ml. of medium	0	0	0	0	75	75	75	75
Streptomycin concentration γ per ml. of medium (before inspissation)	0	6	20	200	0	6	20	200

The medium was inspissated for 65 minutes at 90°C.

Since approximately 50 per cent of the streptomycin is destroyed during inspissation,[5] the final concentrations of streptomycin were approximately 3, 10, and 100 γ per ml. of medium.

Preliminary experiments were performed to determine the concentration of NTC which would not inhibit growth but would give the characteristic color change of an intensity which was easily visualized. A concentration of 75 γ per ml. of medium was found to meet these requirements.

Cultures of virulent *Mycobacterium tuberculosis* var. *hominis*, as determined by guinea pig inoculation, a stock strain of H37Rv, and sputum specimens from cases of clinical tuberculosis which contained acid-fast bacilli by direct smear were used.

The routine procedure for streptomycin-susceptibility determination as recommended by the Clinical and Laboratory Subcommittees of the American Trudeau Society[6] was followed. Portions of the pure cultures were ground with a round-end stirring rod in the bottom of sterile test tubes, diluted with 2 ml. of sterile distilled water, divided into eight equal parts, and inoculated on the eight tubes of medium. Each sputum specimen was digested with 4 per cent sodium hydroxide in a paint shaker for twelve minutes, centrifugalized for thirteen minutes, and neutralized with 8 per cent hydrochloric acid, using 1 per cent phenolphthalein as an indicator. Each specimen was divided into eight parts and inoculated on the eight tubes of medium. All of the cultures were incubated in the slanted position for one week and placed upright for the remaining five weeks. Readings were made at one- to three-day intervals.

The first four tubes in each series represent a streptomycin-susceptibility determination as recommended.[6] The last four tubes duplicate the first four except that NTC, 75 γ per ml. of medium, has been incorporated in them. Seventy-two different specimens were tested. There were 64 valid tests, 2 invalid tests, and 6 specimens which were contaminated. Forty-five of the 64 specimens had the same level of susceptibility to streptomycin, with or without NTC in the medium. The remaining 19 specimens showed differences in their susceptibilities as determined with or without NTC. In 15 instances, the susceptibility to streptomycin was less when tested with NTC in the medium than without it, and in 4 instances the reverse was true.

In figure 1, the comparison of the number of days before growth was first visualized on the NTC tube *versus* the number of days before it was visualized on the non-NTC tube is recorded for each streptomycin concentration. The ordinates indicate the numbers of different specimens which showed growth in the numbers of days indicated under the paired columns. For example, in tubes with no streptomycin, in seven to nine days, 9 specimens with no NTC and 18 specimens with NTC showed visible growth.

As may be seen from figure 1, there is a suggestion of a difference between the mean times for visible growth on the NTC and on the non-NTC tubes. On NTC-containing medium, growth was on the average visualized 0.8, 4.2, 2.1, and 6.7 days sooner on the 0,

[5] Drummond, M. C., Lewis, G. T., and Cummings, M. M.: Dis. of Chest, 1951, *19*, 158.

3, 10, and 100 γ per ml. concentrations of streptomycin, respectively. A statistical calculation of the significance of the differences between the time required for growth on the NTC-containing medium and on the non-NTC–containing medium reveals a standard deviation of 3.58461 from the theoretical mean of zero. The probability of such a deviation is 0.0004, which is highly significant.

It can be concluded, therefore, that the presence of NTC in the medium on the average results in an earlier detection of growth. In 24 per cent this difference was one to five days, in 21 per cent the difference was five to twenty-eight days, in 40 per cent there was no difference, and in only 15 per cent did the growth appear first on the non-NTC–containing tube. In this 15 per cent, there was usually less than a week's difference.

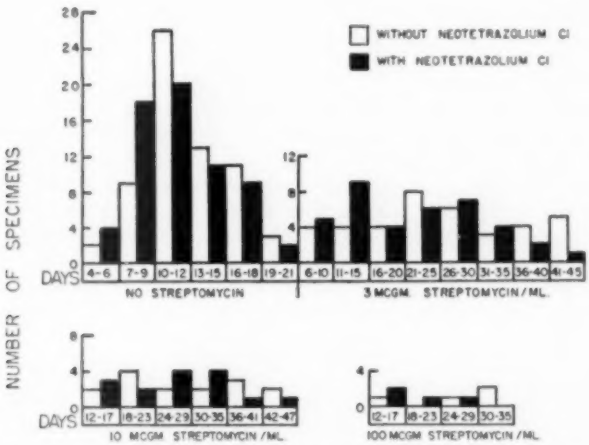

FIG. 1. A comparison of the time interval (days) required for growth visualization on plain medium *versus* the time interval required for growth visualization on neotetrazolium-chloride-containing medium.

In addition to the earlier reading in the presence of NTC, the purple colonies resulting from the reduction of NTC are more readily seen than the pale ivory colonies containing no reduced NTC. This ease of visualization is a recommendation for the use of NTC in the routine isolation and identification of tubercle bacilli. In the usual isolation procedure, four tubes of Löwenstein-Jensen medium are inoculated with each specimen. When no observable growth occurs at the end of the incubation period, a smear is made of each tube which is stained and examined microscopically. By incorporating NTC (75 γ per ml. of medium) into two of the tubes, the time involved in the final step is appreciably reduced, since even the most minute colonies are readily visible on the NTC slant or in the water of condensation. The Pennsylvania Department of Health laboratory has adopted this use of NTC with satisfying results.

It had been expected that the drug susceptibility of a given specimen would be the same regardless of the presence of NTC. In 45 specimens this was true. Nineteen specimens showed differences in their drug susceptibilities, as determined in the presence or absence of NTC.

If a cell population contained many resistant cells, when it was divided into eight equal

portions for inoculation, each portion would contain resistant cells. Upon inoculation the two tubes—one with NTC and one without NTC—containing the maximal amount of streptomycin to which the cells are resistant would both receive resistant cells in the inoculum and growth would appear on both tubes. However, in a specimen containing very few resistant cells, the division of it into eight equal portions would in some instances result in some portions which contained no resistant cell. In this case one of the tubes, either with NTC or without it, containing the maximal amount of streptomycin to which the cells are resistant might fail to receive any resistant cells. This would result in the determination of a different degree of drug susceptibility for the same specimen. As a result of such a random distribution of a small number of resistant cells, which half of the time fell on the NTC tube and half of the time on the non-NTC tube, 50 per cent of the 19 specimens involved should have shown growth on the NTC "end point" tube and no growth on the non-NTC "end point" tube, the reverse being true in the remaining 50 per cent of the specimens. The present results show that 15 of 19 (78.9 per cent) of the specimens grew on the NTC tube and not on the non-NTC tube. This is significantly different ($0.0220 > 2P > 0.0214$) from the predicted percentage on the basis of a random distribution of resistant organisms and suggests that in some way NTC allows organisms to grow in the presence of streptomycin which would not otherwise be expected to grow.

In summary: The incorporation of neotetrazolium chloride (NTC) in routine media used for isolation, identification, and/or drug-susceptibility determination results on the average in an earlier detection of growth, facilitates the reading of growth, and eliminates the necessity for smearing when no observable growth occurs. Nevertheless, NTC in some way allows organisms to grow in the presence of a given concentration of streptomycin which would not be expected to grow at that concentration in the absence of NTC.

Acknowledgment

The writers wish to express their thanks to Dr. C. J. Gentzkow, Director, Bureau of Laboratories, Pennsylvania Department of Health, for his kind cooperation during this study; and to Dr. John R. Preer, Department of Zoology, University of Pennsylvania, for help with the statistical analysis of our data.

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[9] This work was done while the author was a Medical Research Fellow of the National Tuberculosis Association.

TUBERCULIN SHOCK IN MICE INFECTED WITH TUBERCLE BACILLI[1]

In the past, several groups of workers[2, 3] have reported that tuberculous albino mice do not give any indication of existing sensitivity to the tubercle bacillus or to its products. More recently, however, Favour[4] reported specific *in vitro* lysis of lymphocytes from tuberculous mice in the presence of tuberculin. This specific vulnerability of lymphocytes might be interpreted as indicative of the existence of a tuberculin type of sensitivity in tuberculous members of this species.

TABLE I

INTRAVENOUS CHALLENGE WITH TUBERCULIN OF MICE INFECTED WITH VIRULENT TUBERCLE BACILLI OR BCG

MODE OF SENSITIZATION	CHALLENGE INJECTION	DAY AFTER SENSITIZATION	MORTALITY RATIO*
Intravenous injection of 3×10^{-3} mg. (wet weight) H37-Rv	0.3 ml. of a 1:5 dilution of OT	60	0/9
	2 mg. PPD	60	9/9
	1 mg. PPD	60	5/9
	0.5 mg. PPD	60	1/9
Intraperitoneal injection of 10 mg. (wet weight) BCG	0.1 ml. of a 1:5 dilution of OT	10	4/10
	0.1 ml. of a 1:5 dilution of OT	12	5/10
	0.1 ml. of a 1:5 dilution of OT	14	3/10
	0.1 ml. of a 1:5 dilution of OT	17	2/10
	0.1 ml. of a 1:5 dilution of OT	20	1/10
	1 mg. PPD	10	7/10
	0.5 mg. PPD	12	4/10
	0.5 mg. PPD	14	10/10
	0.5 mg. PPD	17	7/10
	0.5 mg. PPD	20	10/10

* Mortality ratio = number of mice dead at 24 hours/number of mice challenged.

In 1952, Hart, Long, and Rees[5] stated, without giving any details, that they had succeeded in fatally shocking tuberculous mice by the intravenous injection of purified protein derivative (PPD). In a personal communication to one of us (W. F. K.), Rees stated that the mice used in these experiments had been infected by the intravenous injection of 6×10^{-4} mg. (dry weight) of *Mycobacterium tuberculosis* H37Rv. Eight weeks later they had developed a stationary "chronic type" of tuberculosis. At this time intravenous injection of 1 to 2 mg. of PPD produced 50 per cent mortality in approximately eighteen to twenty-four hours.

In the present communication, a report is made of the results of intravenous tuberculin challenge in mice infected, respectively, with small numbers of virulent tubercle bacilli or with large amounts of living BCG. These results are summarized in table 1.

[1] This investigation was supported, in part, by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; and from Sharp and Dohme, Inc., West Point, Pennsylvania.

[2] BOQUET, A., NEGRÉ, L., AND VALTIS, J.: Compt. rend. Soc. de biol., 1927, *97*, 1655.

[3] GERSTL, B., AND THOMAS, R. M.: Yale J. Biol. & Med., 1940-1941, *13*, 679.

[4] FAVOUR, C. G.: Proc. Soc. Exper. Biol. & Med., 1947, *65*, 269.

[5] HART, P. D'A., LONG, D. A., AND REES, R. J. W.: Brit. M. J., 1952, *1*, 680.

The results of the experiments with mice infected with small numbers of virulent tubercle bacilli fully confirm the findings of the English workers. The additional experiments show that mice infected with large amounts of BCG develop a sensitivity to Old Tuberculin (OT) and to PPD which is already well developed at two weeks following the infection. Intravenous injection of 0.5 mg. of PPD produces a mortality of almost 100 per cent. The animals infected with small amounts of virulent tubercle bacilli, when challenged at eight weeks following the infection, appear less sensitive since an equal challenging dose of PPD results in a mortality of approximately 10 per cent.

Sensitivity is apparently of the tuberculin type, for shock is of delayed onset. Experiments designed to strengthen this assumption are in progress.

Acknowledgments

The authors wish to acknowledge the help given by Dr. Anne S. Youmans of the Department of Bacteriology, who infected the mice with the virulent tubercle bacilli.

The authors also wish to express their gratitude to Dr. A. C. Bratton and to Dr. Fred Stimpert, Research Laboratories, Parke, Davis & Company, who generously contributed the OT and PPD used in this study, and to Dr. S. R. Rosenthal, Tice Laboratories of the University of Illinois, for the BCG.

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ISONIAZID-DEPENDENT STRAINS OF MYCOBACTERIUM RANAE[1]

The existence of microorganisms with specific nutritive requirements for antimicrobial agents has been established in the case of bacterial mutants dependent upon streptomycin[2] and neurospora mutants dependent upon sulfanilamide.[3] Some resistant strains of *Micrococcus pyogenes* may exhibit increased growth in the presence of sulfanilamide,[4] penicillin, and chloramphenicol.[5] True dependence, as opposed to enhanced growth in the presence of a toxic drug, is relatively rare and therefore of considerable interest.

Strains of *Mycobacterium ranae* completely dependent upon isoniazid may be obtained by isolating colonies from nutrient agar (Difco) supplemented with 2.5 per cent glycerol and 0.1 per cent yeast extract (glycerol-yeast agar), and containing 500 γ of isoniazid per ml. However, the property of dependence is rapidly lost on subculture in media containing 50 γ of isoniazid per ml. or containing no drug. We have not yet tested the possibility of maintaining stock slants on very high concentrations of the drug and at present are unable to subculture dependent strains without loss of this property. When a freshly isolated strain is employed, dependence is clear and unmistakable.

To obtain dependent strains, tubes containing 10 ml. of nutrient broth (Difco) and 0.1 per cent Triton A20[6] were inoculated with the parent strain of isoniazid-susceptible *M. ranae* and the culture grown for four days at 37°C. One-milliliter samples were then plated into Petri dishes containing melted glycerol-yeast agar incorporating 500 γ of drug per ml. Plates were mixed, allowed to harden, and incubated for one week. Colonies were then individually transferred into tubes containing Triton broth and were grown for four days as previously. Each fully grown suspension now consisted predominantly of isoniazid-resistant cells, the susceptible cells having been selectively suppressed by the previous exposure to isoniazid. Approximately 400 of the resistant bacteria from each culture were again exposed in Petri plates to 500 γ of isoniazid per ml. of glycerol-yeast agar, and colonies fished from these plates one week later were resuspended in 2 ml. of Triton broth. After incubation for four days, 0.01 ml. samples from the tubes were streaked on synthetic (M9) and glycerol-yeast agar. Some strains failed to grow on these isoniazid-free media, but on further test were found to produce creamy atypical growth on glycerol-yeast agar containing 50 γ of isoniazid per ml. Isolates from different colonies were not identical in appearance, suggesting that the original colonies were not all clonal descendants of one atypical individual.

Smooth growth without evidence of pellicle was maintained, even after loss of the property of dependence. As an independent check on the identity of the "reverted" organisms,[7] strain Nos. 7, 12, and 15 were found to resemble the parent type more closely if glycerol was omitted from the medium and to be small, faintly acid-fast rods, staining

[1] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[2] Miller, C. P., and Bohnhoff, M.: J. Bact., 1947, *54*, 467.

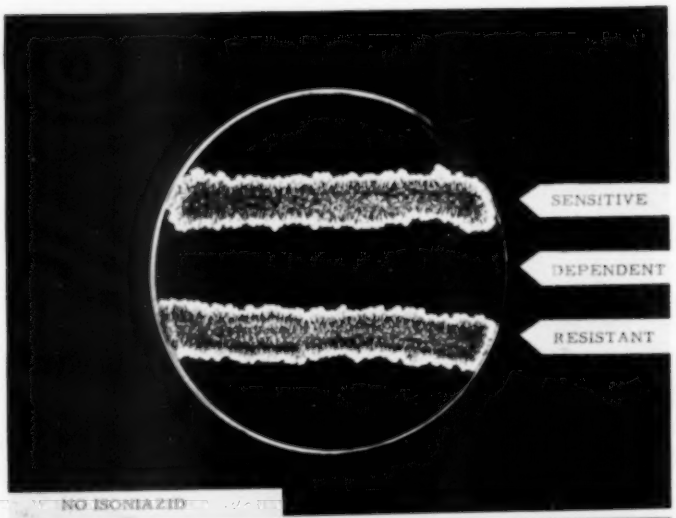
[3] Emerson, S.: J. Bact., 1947, *54*, 195.

[4] Link, T.: Zschr. f. Immunitatsforsch. u. exper. Therap., 1943, *104*, 441.

[5] Szybalski, W.: Genetic studies of cross-resistance to antimicrobial agents: II. Cross-resistance of *Micrococcus pyogenes* var. *aureus* to thirty four antimicrobial drugs. Antibiotics and Chemotherapy (in press).

[6] Proprietary name of Rohm and Haas for a 25 per cent aqueous solution of an alkyl aryl polyether alcohol.

[7] Kindly performed by Dr. A. Brown, Department of Microbiology, State University of New York, Brooklyn, New York.



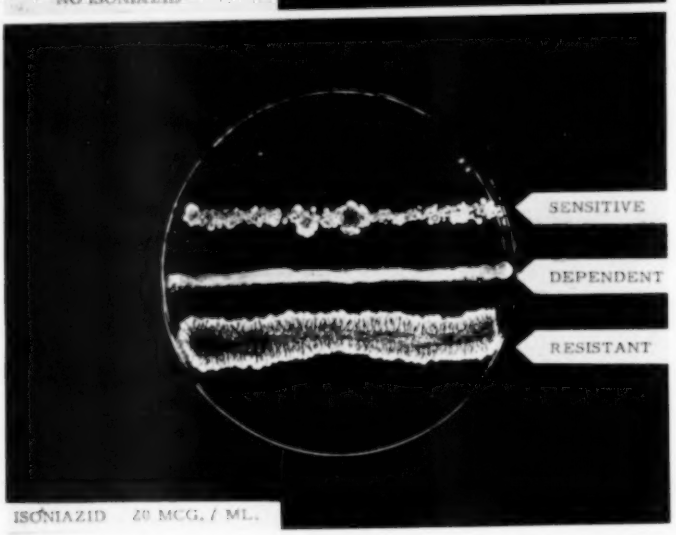


FIG. 1 (Top). Sensitive, dependent, and resistant strains streaked on glycerol-yeast agar containing no isoniazid.

FIG. 2 (Bottom). Sensitive, dependent, and resistant strains similarly streaked on glycerol-yeast agar contaning 20 γ of isoniazid per ml. Note failure of the isoniazid-dependent strain to grow in the absence of the drug.

metachromatically with methylene blue and growing at about the same rate as the parent strain. Moreover, there was no indole production or sorbitol fermentation by any strain, and no growth in glucose broth of strains Nos. 7 and 12. Strain No. 15 was more similar

in colonial morphology to the typical *Mycobacteria* than strains Nos. 7 and 12. Affinity for the gram stain and degree of acid-fastness varied with age of the cultures, but microscopic resemblance to the parent strain was definite on the part of each derived type. Strains no longer dependent remained resistant to isoniazid.

Figure 1 shows the result of streaking 0.01 ml. of three different undiluted cultures from Triton broth onto glycerol-yeast agar: isoniazid-susceptible, -dependent (No. 7), and -resistant. A faint trace of growth of the dependent strain could be seen on the original preparation. Figure 2 represents a simultaneous plating on glycerol-yeast agar containing 20 γ of isoniazid per ml. The upper parent strain, although isoniazid-susceptible, shows some growth of isolated colonies in the presence of the drug because of selective survival of resistant mutants which constitute a relatively large proportion of the susceptible population (figure 2).[1] The dependent strain grows only in the presence of the drug, and the resistant strain proliferates equally on both plates. Nicotinamide in equivalent concentrations may be substituted for isoniazid, but growth of the dependent strains is considerably less vigorous.

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[1] SZYBALSKI, W., AND BRYSON, V.: Am. Rev. Tuberc., 1952, *65*, 768.

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IRREGULAR DISCHARGES

To the Editor of the American Review of Tuberculosis:

I have been thinking some more about your problem of irregular discharge and I am beginning to realize that over here in Great Britain we have no grounds for smug complacency in the matter, because there exist certain basic transatlantic differences between the attitudes adopted towards tuberculosis, infectivity, and treatment. These differences tend to foster the problem on your side and to attenuate it over here.

To explain what I mean I must go back some years. Early in this century the habit of spitting disappeared from this country almost overnight. Public houses were able to give up having sanded or sawdust-sprinkled floors in their bars; and public vehicles, their straw. Why was this? Well, when I was little I was confronted by notices which read quite simply, "Prevention of Consumption. Do not Spit"; and with equal simplicity I read this to mean that, if I spat, *I* was in danger of getting consumption. And so did my contemporaries at my kindergarten. There were, actually, two schools of thought; but the Opposition (headed by a publican's son, I remember), which maintained that it was not only manly to spit but also an indispensable ritual at the end of micturition, was outnumbered by those of us who, with our Nannie's approval, considered that spitting sapped the strength. It was one of those unreasonable magic beliefs like cutting yourself between the thumb and forefinger and dying almost immediately of lock-jaw, or monkeying about with your navel and dying because all the heat of the body would gush out like air from a balloon. A superstition—but how effective! And when one considers the low average mental age of the community—especially where superstition is involved—one wonders whether the same misinterpretation was confined to infants. In public vehicles, the warning had a footnote: "Penalty £5," and many a promising gob must have been swallowed on economic grounds.

Be that as it may, I never appreciated the absence of spitting in Great Britain until I spent six weeks in Washington last May! Now don't get me wrong. I did not find people spitting all over the place, but scarcely a day passed when I did not see at least one person —and curiously enough often a black-coated worker—spitting on to the sidewalk or into the gutter. To Martin Chuzzlewit this would have appeared surprisingly moderate. To me it was just surprising.

Where does this lead to? It leads to the British belief (or wishful thinking) that a person who *knows* he has a positive sputum is very rarely a menace to his fellows. He does not spit and, unless he be a moron or psychopath, he can be taught or trained in a sanatorium never to cough openly, never to kiss children, and other simple hygienic precautions.

Between 1900 and 1950, many hundreds of patients were still sputum positive at the time of their discharge from this sanatorium, because there were no means of ever making them negative. In a recent follow-up, only two patients were traced as having infected other members of their families, and in each case the infector had been negative at the time of discharge and had been led to believe that he had remained sputum negative for some years. Both patients, in fact, were suffering from unsuspected recrudescence of their disease.

The British assumption is that tuberculosis is spread by the *undiagnosed* "open" case—the schoolteacher, the "bronchitic" relative, the factory hand, or the bank clerk. Hence

the primary function of our mass radiological surveys is to bring to light the sputum-positive case, so that thereafter he knows he is, and has been, a potential source of danger to the community and that he need be so no more.

Convalescent sanatorium patients who are doomed to remain obstinately sputum positive are reassured, "If you take these simple hygienic precautions, your children run far less risk with you than they do every time they ride in tube, train, tram, or bus. All the same, before you go home to them, get them Mantoux-tested at the Clinic, and if they are still negative, have them BCG-ed."

You see, we don't utterly disregard the dangers of infectivity, but by your mask-wearing standards we undoubtedly play it down. In our institutions, should you see any member of the staff wearing a mask, you may be sure he is either a laryngologist going into action, or he is suffering from a head-cold in its infectious stage and is heroically sticking to his job instead of segregating himself.

The foregoing now helps to throw light on a basic difference of attitude between U. S. A. and Great Britain regarding what is meant by treatment. I gather that in U. S. A. treatment is not regarded as complete until the sputum is persistently negative on smear and culture. Indeed, is it not a penal offence in some states to be at large with a positive sputum?

Our goal is less definitive and less ambitious. True, whenever we think we can make someone tuberculosis negative, we try to do so, unless the procedure is likely to turn him into a respiratory wreck. We do not like bringing too much pressure in favor of surgery to bear on a reluctant patient, even when he is a schoolteacher who will automatically lose his job if his sputum remains infectious. Too frequently his treatment turns sour and we wish we'd never touched him. One cannot exclude hunches, the pneumo-psychosomatic element, and other imponderables from one's calculations; and many factors other than radiological shadows and sputa seem to influence our decisions. A business or family crisis may need urgent attention and appear more threatening in its consequences to a family constellation than a positive sputum.

It seems evident, therefore, that in our softness we discharge quite a number who in another discipline would become "irregular discharges"; and this surely must be the main reason why that particular problem arises less frequently with us.

Again, what are our patients' motives for undergoing treatment? I am not sure, but in Great Britain I think it is chiefly to avoid premature death. Judging by Dr. Harry Wilmer's writings and the many varieties of "How To" books dished out to your patients, U. S. A. phthisiologists seem to play down the idea that tuberculosis could possibly kill anyone who has come under their care, and the Big Appeal seems to be "Stop Being a Leper."

In short, our differences are differences in emphases and their disposition. In Great Britain we play down infectivity. In U. S. A. you play down death. Which is better and which is worse I am not in a position to argue. And who is? They both seem to work in their way.

THE SANATORIUM
MUNDESLEY
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Sincerely yours,
GEORGE H. DAY

June 1, 1953

American Trudeau Society

Medical Section of the National Tuberculosis Association

Seminar on the Therapy of Miliary and Meningeal Tuberculosis

Report of seminar held at the annual meeting of the American Trudeau Society, Los Angeles, California, on May 20, 1953.

Dr. Paul A. Bunn, *Chairman*

DR. BUNN: Welcome to the panel. We're labeled here as a group of experts to talk about the therapy of miliary and meningeal tuberculosis. Unlike some of the previous panels of yesterday and the day before, the order is going to be changed. Rather than throw the seminar open to immediate questions, I thought that perhaps it might be proper to review certain problems in this disease or these diseases, and that during these opening remarks I would pose several questions to the panel. I must admit that it's been a little bit rehearsed, not altogether fortunately, because panel members are not in universal agreement on the way in which the questions should be answered.

Since 1946 we've had a reasonably good agent for treating tuberculosis. If we were using this agent properly, there probably would be no reason to have this seminar in the first place. The results of therapy, of meningitis in particular, are still rather embarrassing and it is for this reason primarily that the seminar was arranged. Reviewing the history of the treatment of meningeal tuberculosis is fascinating as it always is in the general field of tuberculosis. Reviewing certain outstanding kinds of treatment in the past, I think, allows us to make just one statement. Interestingly enough, back in the thirties, tuberculin was being used intraspinally for the management of patients with meningitis. Except for that one item, actually originally described by Dr. Koch before the turn of the century, we have no carry-overs on how to manage the patient with tuberculous meningitis prior to the advent of streptomycin. I believe that Dr. Hinshaw and his cohorts at the Mayo Clinic deserve first credit for describing the use of streptomycin in the therapy of patients with both miliary and meningeal disease. The original report came out in 1946 and the subsequent good descriptions of treatment regimens were outlined by Dr. Lincoln and Dr. McDermott. A reasonable program was described in 1947. The survival rates described on a large scale, first perhaps by the English and subsequently in this country by the Veterans Administration's cooperative study, indicated that about 20 per cent of the patients with tuberculous meningitis could be cured. And perhaps we can use that term without even putting quotation marks around it. Since 1947, when these figures were first described, there really has been no change in the mortality figures; from 1947 at least through 1951, we were still saving the same small percentage. This is the part of the treatment program, it seems to me, that remains embarrassing, in five years we have not accomplished very much even though we have had streptomycin and, since 1947, a variety of other compounds and tricks and variations in their administration to help improve the mortality figures.

It seems to me that these observations require that three direct questions should be directed to the panel.

The first and most important of these: Why haven't we done better in the treatment of tuberculous meningitis in the past six years? Secondly, as one reviews the literature from England, from Europe and from the United States, universally the English and the

Europeans have done better, impressively better, curing in the neighborhood of 40 to 50 per cent of individuals with the disease and its complications. Very few series in this country in adults ever approached that figure even at the six-month observation period. Similarly, in reviewing the literature, it is observed that the therapy seems to be more successful with children than with the adults who have the disease. Now these three questions then are: Why the discrepancy between European and American figures? Why do children do better with treatment? And, most importantly, why have we made no inroads on the mortality figure, which is not good.

It is perfectly evident, at least to me, though after yesterday I am not sure about Dr. McDermott, what is the basic ingredient for the therapy of meningitis, but I think at least we have to consider still that streptomycin is the basic one. Whether or not we should add other drugs to the therapy of this usually fatal disease I think will be discussed also today. I think it would be foolhardy for this group, or actually the individuals in the audience, to consider that we have to wait for a new drug to do better.

I am reminded very much of our experiences with pneumococcal meningitis, for example. Prior to the advent of the sulfonamides, the disease was fatal in 90-odd per cent of all cases. With the development of certain of the sulfonamides, particularly sulfadiazine, the mortality rates were reduced to approximately 75 per cent; and in 1942 and 1943, when penicillin was first being used, mortality rates were further reduced to approximately 50 per cent. Then, like the history of tuberculous meningitis from 1942 to 1949, we progressed not at all until 1950, the 50 per cent mortality rate being more or less common. With imagination and actually with the elimination of certain agents and tricks of therapy, Dr. Dowling first demonstrated that with the use of penicillin alone in a different way the mortality rate could be reduced significantly. Actually now in the treatment of pneumococcal meningitis, in most series the mortality rate of approximately 30 per cent has been accomplished, not with new agents or new tricks, but with imaginative prescription of the single best agent. There is a pharmacologic reason perhaps why that program works, but at least it brings attention to the point that we don't have to await a new drug or a new treatment program to hope to do better in the treatment of tuberculous meningitis.

Rather than try now to review strictly mechanical details of treatment, it seems to me that, if we are going to use our imaginations and try to do better with the agents available, we have to do a little review of the total disease picture, hoping to fit treatment with a clearer understanding of the pathologic processes going on in the adult or in children.

I am very proud of this panel with the possible exception of the fact that we have no neurosurgeon; there are very few neurosurgeons at this meeting. These experts, I think, are equipped to talk in an intelligent manner on every phase of tuberculous meningitis. I think we should introduce them. At my left, Dr. Kenneth Smith, who is the chief of the Pulmonary Disease Section of the Veterans Administration Hospital here in Long Beach; next is Colonel Carl Tempel, who is chief of the Medical Service at Fitzsimons Army Hospital in Denver. To my immediate left, Dr. Oscar Auerbach, who is chief of the Laboratory Service at the Veterans Administration Hospital, East Orange, New Jersey, and associate professor of pathology at the New York Medical College. To my far right, Dr. Dwain Walcher, who is associate professor of pediatrics in Indianapolis; Dr. Edith Lincoln, who is professor of pediatrics at New York University and chief of the Children's Chest Clinic at Bellevue. And to my immediate right, Dr. Walsh McDermott, whose comments yesterday have perhaps deserved some apology today.

DR. MCDERMOTT: You mean from the panel?

DR. BUNN: Yes, the Chair in this case not dissenting.

And finally, I think it is very important that we do one thing today if nothing else. I think the idea of discussing miliary tuberculosis and meningeal tuberculosis in the same breath is not proper. The two disease processes, it seems to me, are completely separate things; both are serious and often fatal complications of tuberculosis, but in no sense are they synonomous complications. I think it wise for several reasons to separate miliary tuberculosis. Like staphylococcal sepsis or other kinds of sepsis, we do reasonably well in the management of this kind of disease, tuberculo-sepsis, if you will. And I doubt if we will talk very much about miliary disease today, except as it is a part of meningitis in some instances. The more leisurely and exceedingly slow-to-resolve tuberculous meningitis is a much greater challenge and for that reason we will spend far more time with it. On occasion it is associated with miliary disease; I think it reasonably unimportant. The primary discussion will be centered about meningitis. If the audience or the panel disagree with that, we would be happy to argue the point, perhaps. But the program is full and, if we can come up with a straight answer on how to improve our therapeutic management of patients with tuberculous meningitis, we will indeed have done something I am not sure we are going to do.

Perhaps the first question, although I have posed three up to this point, I think the first question is really—How important is tuberculous meningitis in the over-all problem of tuberculosis? Is it a rare disease? Are we seeing more of it? Are we seeing less of it? The incidence, it seems to me, is such that we deserve to have a few comments—I think Dr. Auerbach can do it—on just how common this problem is. Dr. Auerbach, if you will lead off for a couple of minutes, I think it would be good.

DR. AUERBACH: In an institution devoted chiefly to tuberculosis where each individual is admitted to the institution, whether a child or adult, with an active form of tuberculosis, the incidence of tuberculous meningitis at autopsy is approximately 4½ per cent. If you prefer to break that down, you find that in the small number of deaths of children, which is approximately 100, the incidence of tuberculous meningitis is approximately 42 per cent. In a much higher autopsy rate, over 2,000, the incidence of tuberculous meningitis in adults is 2.9 per cent. Two and nine-tenths per cent of all adults who die of tuberculosis have as the cause of death tuberculous meningitis.

DR. MCDERMOTT: In the United States?

DR. AUERBACH: In a hospital for tuberculosis where I have gathered the information.

DR. MCDERMOTT: Well, I think perhaps that deserves a comment. Your figures, of course, are weighted, aren't they?

DR. AUERBACH: Yes they are; it's a tuberculosis institution where the only persons admitted are those who have an active form of tuberculosis before they get into the hospital.

DR. BUNN: Dwain, do you have any comment to make on that? Is it really that common a disease?

DR. WALCHER: Paul asked me to make some comments about the incidence of tuberculous meningitis in both children and adults. I shouldn't be criticizing the figures which Dr. Auerbach has given now, for I found it impossible to answer the question with any degree of security because I realize the loaded characteristics of these figures and, in discussing this situation with some of the group this morning, I was very much interested in the incidence which Colonel Tempel tells me he has noted in the army. I think, if I am quoting your figures correctly now, you see about one case of tuberculous meningitis per 1,000 admitted to the army hospital with a diagnosis of tuberculosis. Is that not correct, Colonel?

COL. TEMPEL: That was miliary and meningitis, but they were nearly all meningitis; you were right.

DR. BUNN: One patient out of a hundred who came into the hospital with active disease had meningitis.

COL. TEMPEL: Our admission rate would be one per 100 per year.

DR. BUNN: One meningitis per hundred?

COL. TEMPEL: Yes, that's true; but our admission rate for tuberculosis in the service is about one per thousand men per year.

DR. WALCHER: I think that's a great hazard which we always have to consider when we talk about incidence of any of these more serious complications; perhaps the most startling one to me is to talk about measles and measles encephalitis, and I find figures in the literature where some man says it occurs once in every 460 cases and the next man says once in every 15,000. So, if you get such wide variations between one in 500 and one in 15,000, it is very obvious that the figures for each individual have been loaded in some way.

DR. BUNN: I think we can at least admit that the problem is a significant one; that the incidence of the disease is such that we have to consider it a potential cause or complication of every patient with tuberculosis.

DR. MCDERMOTT: I think it should be pointed out that the figures which have been cited apply to the United States. In areas with less highly developed technology in which the prevalence and incidence of tuberculosis are very much higher, actually the incidence of tuberculous meningitis or indeed miliary tuberculosis probably provides the best index of the amount of tuberculosis in the community; and in places other than the United States, England, Scandinavia, and, to a certain extent, France and Italy, I dare say that the incidence of tuberculous meningitis and miliary tuberculosis is much higher than has been cited, and is even higher than Oscar's weighted statistics.

DR. BUNN: More than 3 per cent you mean?

DR. MCDERMOTT: At least that.

DR. BUNN: It makes the problem all the more acute.

To leave that for the moment, I think I would like to ask Oscar one more specific question. As any feature of medicine, we generally have to get back to the background of the disease process.

I think first of all we've got to decide whether or not we're treating a hopeless situation, pathologically speaking; if the tubercle bacilli in the first few days of its existence in central nervous tissue do irreparable and irreversible damage, then again there would be no reason to have this panel. For that reason I'd like to ask Oscar if he would tell us just a little bit about how reversible a disease we are actually treating. Can it be done under ideal situations?

DR. AUERBACH: I think we definitely can treat it under ideal conditions and, in the first phase of the disease at least, the condition is completely reversible. The tuberculous meningitis that we see in the early onset is that of an exudate which lies between the pia and the arachnoid and is composed of serum fibrin and neutrophils. As all of us know, that is an exudate similar to any exudate in the body and, if it were in the alveolar spaces, we'd call it pneumonia. We know well that pneumonias are reversible with or without chemotherapy. So, from that point of view it certainly is reversible. But, soon after that, and from the untreated cases that we know, we see evidence of necrosis which develops in the exudate by the fibrin swelling in the cells undergoing necrosis, and around that necrotic zone is an area of repair, a typical area of repair which we know is tuberculous granulation tissue. Now, even in the cases which we know have lasted for three weeks, we've been impressed with the small amount of necrosis and the small amount of repair which

we see in the tremendous exudate. All of you recall that the typical appearance of the exudate was a gelatinous area at the base of the brain and along the sylvian fissures, and that we were able to make a gross diagnosis by the presence of tubercles along the blood vessels and these tubercles were intimately adherent to the vessel walls and represent tubercles in the adventitial portion of the vessels; so I think in the first few weeks, at least from the gross and microscopic findings, that the answer is yes, the condition is reversible.

DR. BUNN: Generally you would use that figure of two or three weeks, Oscar, as being the limit of time that one has to make a clinical diagnosis and expect total recovery, within that period of time?

DR. AUERBACH: Yes, I would. That is based on the fact that in the great number of cases we saw before the era of chemotherapy there was so little necrosis in the exudate at that period.

DR. BUNN: For those of you who have not read Oscar's paper on this, I really believe it is a masterpiece of writing about tuberculous meningitis, published in the AMERICAN REVIEW a year or so ago. There are two papers: one on the treated group and one on the general considerations of the disease process.

Dr. Walcher, do you have any more comments on the pathological background—you have studied this in children? Would you say it was any different from what Dr. Auerbach has described?

DR. WALCHER: I think not, Paul.

DR. BUNN: Well, this gives the clinician a chance, then. We have obviously at least two or three weeks in which the disease process can be expected to resolve under ideal treatment.

Dwain, can you make the diagnosis of the disease in that time in children?

DR. WALCHER: Yes, we most certainly can.

DR. BUNN: Do you?

DR. WALCHER: Yes, we do.

DR. BUNN: Now, tell us a little bit about the clinical picture of early tuberculous meningitis in a human who can't talk, or does so unintelligently.

DR. WALCHER: Well, I said we can make the diagnosis. We do it most infrequently actually, for most of the children whom we admit to our own hospital come to us when they have had their disease somewhere within the latter part of the third week of their illness which throws them far into the period when one can anticipate good recovery from any of the therapeutic agents we are now using. We are most happy if we can see a child whom we consider in the early stage of tuberculous meningitis. We generally group them into the early, the moderate or the medium, and the late stages. Actually those children with the early form of tuberculous meningitis are those children who are irritable; they may be running a low-grade fever of 100, 101°F. We sometimes find that they have difficulties with their feeding. There may be vomiting on occasion. There may be more than minor regurgitation to losing large amounts of the fluids which they take in. There may be nothing else significant in these infants. Unfortunately, this is insidious. It does not draw to the attention of the parents, and so often to the family physician, the fact that there is something serious going on. Therefore, it is allowed to continue until frank neurologic findings are present. The child later slips into a semicomatose and perhaps a comatose state. Fortunate, I think, is the child who has convulsions, for that usually shocks the family into the realization that here is something which isn't quite normal and the child had best see the physician.

In the past two weeks we have had two children admitted to our institution; one who

has had tuberculous meningitis for four weeks and the other for three weeks, unrecognized as such. Those are the two admissions we have had in that interval of time. Neither of them came in early in the course of the disease. It's most uncommon for me to see it in the early stages.

In the first series which we reported the average duration of illness was 19 days before the onset of actual therapy in these children. Dr. Lincoln's very good review of the course of meningitis showed that these children were ill about 19½ days on the average; some one else said 20 days prior to their demise. So you see we have on the average between a half day and one day of therapy. Our figures are very poor. I say this with some apology for our end results, but I think it does offer an explanation. It points up the extreme necessity for the first person who sees this child to be aware of the possibilities of tuberculous meningitis. Often that first person is a member of the family, with a great lethargy in the recognition of these symptoms. I think that carries it clear back to tuberculosis in the adult, which is where the child picked up his contact.

DR. BUNN: Dr. Tempel, do you want to make any more comments about diagnosis in the adult other than those statements? We don't want to spend too much time on early diagnosis. I think it's always a proper philosophy to treat it early once you've made the diagnosis.

COL. TEMPEL: You want me to give a little of the picture we see at our hospital?

DR. BUNN: Yes, very briefly.

COL. TEMPEL: I might say, to introduce this subject, that since 1946 there have been 82 patients with miliary or meningitis. Of these, 61 were meningeal, so that my evaluation would be based on the observations on that group. Our age group was 18 to 52, with an average of 26. Negroes to white ratio, two white males to every one Negro. In our pulmonary series it's a four to one ratio at the same institution so that the incidence is a little higher. And speaking of complications in the group as we see them, half of our patients had complications of adenitis, bone tuberculosis, genitourinary tuberculosis, cavitary tuberculosis, or pleural effusion.

DR. BUNN: Do you ever see them without any such focus?

COL. TEMPEL: Oh, yes.

DR. MCDERMOTT: Did it make any difference whether they had miliary or didn't, Carl?

COL. TEMPEL: At first it did. About half of our cases had miliary associated with the meningitis.

DR. MCDERMOTT: And they did worse?

COL. TEMPEL: I would say that was a reasonable answer prior to our more recent therapies. Ten per cent of these folks had multiple complications, 10 per cent had one or more of these lesions. The duration of their illness—all of these were picked up in the first month and it was usually within the first three weeks. That's understandable in a group of young men on military duty where we have an opportunity to see them rather promptly if they have headaches, nausea, vomiting, which brings them to a physician perhaps a little sooner than a civilian. I think one of the interesting things is the spinal fluid picture and the clearing of the X-ray. Of course, this is a progress report rather than a picture. I won't go into all the details of our findings, but the cerebrospinal fluid in our series cleared in nine to eleven months and the range on that was four to twenty-three months. I thought that was a rather interesting aspect of our study. In those having miliary tuberculosis, clearing occurred at two to nine months, with an average range of five to seven.

DR. BUNN: I think one of the major problems in the early diagnosis is not only the clinical picture but the terrible situation where the spinal fluid may be abnormal, yet

there are no organisms found, and in the presence of a picture of infection. How important is it, Dr. Lincoln, in your children to find the organism before you expose this child to long, expensive, and difficult therapy? Do you await the finding of the organism?

DR. LINCOLN: I don't think you can ever afford to wait to find the organism.

DR. BUNN: Is there something characteristic about the spinal fluid alone without the organism that will make you treat a patient?

DR. LINCOLN: I think the spinal fluid in tuberculous meningitis is very characteristic. It usually is a clear fluid with a moderate increase in cells, somewhere between 10 and, say, 300, which is usually mostly lymphocytes but in very acute cases, in fulminating cases, it may be largely polymorphonuclears. The sugar is usually the most characteristic thing about the fluid of tuberculous meningitis and is usually low; but a sugar just about 40 or even higher should not deter one from the diagnosis, particularly if it is lower on a second examination. The protein is usually moderately increased. It is customary to think of a great many other things in the differential diagnosis of tuberculous meningitis, but actually there are very few things which have that picture and most of them are very rare. The one with which we ourselves have most difficulty is chronic *Hemophilus influenzae* meningitis, but even then it should be possible to find the organisms within twenty-four hours. If no other cause for the changes in the spinal fluid are found within twenty-four hours, especially if one has a positive tuberculin test, X-ray evidence, and so on; but even without those I think one should go ahead and treat the case as tuberculous meningitis.

DR. BUNN: Dr. McDermott, do you have a comment?

DR. MCDERMOTT: Yes, I have. I would certainly agree with everything that Dr. Lincoln and Dr. Tempel have said and particularly with what Dr. Lincoln just said about the characteristic cerebrospinal fluid findings, including the low sugar. But she also said that upon occasion one may have a normal concentration of sugar. What I would like to point out is that the physician faces a dilemma in adults who present these cerebrospinal fluid findings of nonpurulent meningitis with a normal concentration of sugar and with no other evidence of tuberculosis. On the one hand, it is agreed we wish to treat at the very earliest moment; on the other hand, it all depends upon which treatment one uses. If one is to use, let us say, the intrathecal administration of streptomycin, to start one's assistant resident with lymphocytic choriomeningitis on a program of intrathecal streptomycin with its very high incidence of deafness is not a situation which one faces with equanimity, so that we could use better and more rapid methods. In situations in which tuberculous meningitis has a very high prevalence, then the problem is easier. In a situation which occurs among adults where the prevalence is not very high, then the physician faces a very obvious dilemma and a dilemma to which I see no solution.

DR. BUNN: I think there will be a solution in another fifteen minutes, Dr. McDermott, if you're patient.

DR. MCDERMOTT: I have all morning.

DR. LINCOLN: I think there are two points about that. One, the obvious one, that intrathecal therapy may now be unnecessary; but the other one, the fact that even if you wait twenty-four to forty-eight hours you usually have your differential diagnosis of lymphocytic choriomeningitis, because, even in the case which starts with polymorphonuclears, it will change very quickly.

DR. MCDERMOTT: How about the sugar, though? Haven't you seen sugar stay about 35, say for four or five days?

DR. LINCOLN: Yes, but it usually tends to fall, whereas in the viral meningitides they usually tend to increase or to stay at a certain level.

DR. WALCHER: Paul, may I have one moment? I want to emphasize again this most important point of family contacts. In a series which we have studied in considerable detail and pursued rather far with respect to family contact, we find 75 per cent of the contacts come within the immediate family and, whenever possible, when the parents come into the admitting room with the child when we suspect tuberculous meningitis, they don't leave the institution until chest films have been taken. I'd just like to point out that one father who had only a slight cigarette cough had a huge cavity and his wife had tuberculous pneumonia. Neither of them had seen a physician. The child died with tuberculous meningitis within two weeks. It is important to consider family contacts as our source, and it's been a tremendous help to us in making the diagnosis.

DR. BUNN: I'd like to speak as the prerogative of the Chair about looking for the tubercle bacillus. It seems to me that in those instances where the tubercle bacillus is found on the first day, one can relax in the responsibility of how abrupt the treatment, or how hard it is, or how long it's going to be. Now, there's pretty clear correlation between the number of minutes spent looking at a slide and the number of positive slides found. Dr. Riley, for example, in his very recent report in the REVIEW, suggested that in the majority of cases tubercle bacilli were not found on direct smear. I wish to take exception to that statement because I think in the vast majority tubercle bacilli will be found on the very first smear if looked for carefully enough. Now, one doesn't like to draw arbitrary limits on how long or how assiduously you should look at a smear of spinal fluid, but it seems to me that, if they're not found earlier, a minimum of two hours should be spent looking over a slide of spinal fluid in the hopes of finding a tubercle bacillus. Every one agrees that it takes too long for the cultures to grow out. The one opportunity then is to examine the spinal fluid by smear. The proportion of positives, even in early cases of meningitis, will exceed 50 per cent and perhaps be as high as 80 per cent in our individual series by that technique; and there are no tricks, as far as I'm aware, to make the cells show up better. Now, Ken, you've gone through the VA series very recently; do you have any ideas on this diagnosis from spinal fluid alone?

DR. SMITH: No; frankly, I'd like to add that I quite agree with you that the fluid should be studied assiduously on the smear. I have nothing more to add. I quite agree with Paul Bunn.

DR. WALCHER: Paul, should you spin the spinal fluid or wait for a pellicle to form?

DR. BUNN: We do both, as a matter of fact. We separate the spinal fluid. We found the greatest success, though, in the spun specimen, spun at a high rate of speed.

DR. WALCHER: How high?

DR. BUNN: Oh, better than 2,000 and perhaps closer to 3,000 if you've got a machine to do it. Unfortunately, the tubercle bacillus does not always settle at lower speed, such as 1,200 to 1,500. And indeed you might find them better in the supernatant and not in the sediment. And, consequently, high speeds for long periods are essential if you're going to spin them. Finding them in the pellicle automatically means that the protein concentration in that spinal fluid is going to be reasonably high or else the pellicle will not form; in many cases of early tuberculosis the pellicle formation is not present, at least for twenty-four and sometimes thirty-six hours. For that reason, it seems to me, the spun sediment is far more important than waiting for the pellicle to form and to smear it. Now, perhaps others on the panel would not accept those statements. That's my personal opinion and that is the way we do it.

Dr. Lincoln, would you comment on that, on the preparation of the spinal fluid for the finding of the organism?

DR. LINCOLN: No, I think the spinning at high speed is the easiest way to find it. We

have not been able to get people to spend enough time looking for it. When I was an intern we weren't allowed to make a diagnosis of tuberculous meningitis until we found the organism, which sometimes took six hours.

DR. BUNN: I'd like to make it very clear that, although the differential diagnosis in children might be easy, in adults, with the variety of encephalitides that we see (including choriomeningitis), the differential diagnosis on spinal fluid is exceedingly difficult if one considers protein and cells and sugar on one specimen and even on specimens twenty-four hours apart. There is actually no clear-cut indication for a diagnosis of tuberculosis on spinal fluid findings, alone, it seems to me; it takes much more unless the organisms are found. All the more reason then to find the organism. I think rather than spend more time on that, unless other members wish to argue the point (and I hope they don't) we've got so many agents, and gimmicks, and gadgets here to talk about specific therapeutic problems—I think we'll go directly to that.

Initially, I had thought perhaps each of the panel members ought to be able to describe how he or she treats an individual case of tuberculous meningitis. I'm sure, if we did that, we'd end up with about five different programs; and that probably there would be very little among them that would have a common denominator except streptomycin. Consequently, I would feel a little bit more at ease in trying to moderate these differences, if we talked a little bit specifically about agents first and then later, perhaps, dosages and how they should be given. And to do that I think we should review exactly what we have done in the majority of instances in a larger single series in the adult. Ken, do you want to review what you've found out about the treatment of the VA series—in its initial phase it was pure streptomycin—and then go on to the added drugs, if you have that information easily available.

DR. SMITH: You all know that Dr. Paul Bunn studied and reported the first 100 cases of miliary and meningeal tuberculosis treated with streptomycin alone. His first report dealt with survivors four to fourteen months after the start of therapy and, for those who had meningitis alone, 37 per cent were then survivors. Two years later he made a follow-up study in which it was found that the survivors had dropped to 18 per cent.

Recently, I had the fortunate experience of checking up on these known survivors to find out how many had lasted for five years. The gross rate dropped to 12 per cent, but none of these additional deaths were due to the original tuberculous meningitis. I think it therefore significant that the two-year follow-up was the correct one, and I suggest that it be borne in mind in future comparative studies. Now, in the total VA experience brought up to date, there were 121 cases treated with streptomycin alone, and at the twenty-four-month post-treatment the survival rate was 26 per cent. Now I must disagree in part with Dr. Bunn's comments that we have not improved because I think we have. We had 89 cases treated with streptomycin and PAS reviewed twenty-four months after treatment; we had 44 per cent of survivors.

DR. BUNN: Now, what year is this, Ken?

DR. SMITH: This is brought up to 1953, that is, this report was made in February, 1953.

DR. BUNN: So you're answering the first question that I posed to the panel, why haven't we done better since 1947. You're saying that we are doing better.

DR. SMITH: We are doing better because we've combined two chemotherapeutic agents.

DR. BUNN: Have you had enough experience in the last eighteen months to really say that you're doing better?

DR. SMITH: Yes, sir.

DR. BUNN: How much better?

DR. SMITH: In this group, 25 to 45 per cent.

DR. BUNN: We're catching up with the English, almost!!

DR. SMITH: Now, as for the third drug, we can't of course give you twenty-four months as our follow-ups. We'll have to go back to six months for comparison. With streptomycin alone, at six months only 49 per cent survived. When we combined the two drugs, streptomycin and PAS, the survival rate was 63 per cent. For the few cases of streptomycin and isoniazid that we have available, 7 at the time of this compilation, the survival rate was 80 per cent.

DR. BUNN: At what time?

DR. SMITH: This is at six months. Streptomycin alone, 50 per cent; streptomycin-PAS, 60 per cent; and when you use isoniazid with streptomycin, 80 per cent survive.

DR. BUNN: But only 7 cases in that last category?

Carl, have you any better figures than that, to see if we are doing better? I'm afraid my first question is being answered as a complete surprise.

COL. TEMPEL: Yes, I think we can be more optimistic than you were.

DR. BUNN: I'm not a bit optimistic. Even a 60 per cent survival rate is not good enough.

COL. TEMPEL: In 1946 and 1947, we treated 20 cases and we lost all but 3, actually 85 per cent. From that period up to about seven months ago I added up the figures, and we have saved 63 per cent of that group. Now, since February, 1952, we initiated 300 mg. of isoniazid combined with streptomycin-PAS, on which we have 14 patients under treatment. There's only one death in that series and most of the patients are doing very well, so I'm a little optimistic.

DR. BUNN: How many of the 14 have been followed for six months?

COL. TEMPEL: Oh, I should say at least 8 of them.

DR. BUNN: You got one death out of 8, then. What we're talking about is six months—arbitrary division here? We can't count the fellow who started treatment yesterday and is in your series.

COL. TEMPEL: Well, I'm not counting any whom I haven't followed for at least six months. And I think that's pretty good, isn't it, one out of 14.

DR. BUNN: You see, Dr. McDermott, yesterday you made the comment that you'd like to use good agents singly, not throw them all together in the same patients. Perhaps you might be willing to make a comment about that. It seems to me that with the figures just presented—Let's go to the pediatricians here. Do they do any better with isoniazid with streptomycin, or with three agents, than they did in the streptomycin period alone, or with Promizole, Dr. Lincoln?

DR. LINCOLN: Well, I have to agree with you that during the first five-year period we couldn't better our records. We've been curing 2 of every 3 cases since we started streptomycin-Promizole. Of course, we've used combined therapy from the beginning. We've used streptomycin-Promizole from our first case and we've had 48 cases with a total of 18 deaths, 16 due to meningitis and 2 accidental. That was up to February, 1952. Since then we've had 15 cases of meningitis, 11 of which have been followed for more than six months, and we've had only one death. That is with the continuation of streptomycin intramuscularly, dropping streptomycin intrathecally, and continuing Promizole and adding isoniazid by mouth.

Dwain, do you have any comment on that? You've had an unfortunate series because all of yours have been in coma, so that actually how good are you doing with isoniazid over streptomycin in the four-plus case?

DR. WALCHER: We have had 11 patients on isoniazid and we have lost 3 of those

11 so far. They all died within two weeks after their hospitalization. The other 8 are alive and I have the feeling they will survive. I'm not certain they are going to be able to get through school. They certainly have a severe amount of cerebral damage.

DR. BUNN: Well, now, Walsh, these are rather overwhelming figures, better actually than I had realized. You still defend that statement you made yesterday that you should not use two powerful agents together?

DR. MCDERMOTT: Well, in the situation which I was talking about yesterday, I was talking about chronic pulmonary tuberculosis.

DR. BUNN: We were talking about young nurses just converting to tuberculin.

DR. MCDERMOTT: In either of those two situations, it is my personal belief that it is a mistake to use isoniazid and streptomycin together. I have no objection to the use of PAS with streptomycin. Moreover, I made no statement to the effect that PAS might not have antituberculous activity; all I said was that I'm not convinced it does anything about the emergence of streptomycin resistance.

Now, in so far as the treatment of meningitis is concerned, I think that one point strikes us, Carl Muschenheim and me, very forcibly; that is, when one is talking about miliary tuberculosis, or laryngeal tuberculosis, or perhaps even tuberculosis of the lymph nodes, one is talking about reasonably identical biologic units; and you can take 14 patients of this category and 14 patients of that category and put them on an IBM card and come up with something. Now, when one is talking about tuberculous meningitis, one is talking about children and adults in the first place; one is, as I'm sure Oscar Auerbach will agree, talking all the way from something the size of a golf ball in terms of the tuberculoma to a reversible process of fibrin and polymorphonuclear neutrophils on the other. And there is no way that the clinician can tell whether he's got a golf ball in there or whether he's got what might be called, to all intents and purposes, a serous meningitis. As a consequence, I think that drawing therapeutic inferences about tuberculous meningitis with the sole exception of did they die or didn't they die on a given drug—from that point on, one gets on very dangerous ground. The only way out of that is obviously the sort of stuff Ken Smith is doing and that is to get very large series, so the golf balls and the serous meningitis and the infarcts of the brain, with the arteritis and all that sort of thing which Oscar has described, can be evened out.

Now, in so far as the use of two drugs is concerned, Paul, I am heartily in favor of the use of two drugs in the treatment of tuberculous meningitis. I think that on any regimen of therapy, no matter what, we are going to have some deaths from tuberculous meningitis. If we have a death and we use drugs A and B, and we didn't use A, B, and C, we're going to wish that we had given C. So that from an ethical standpoint, if from no other, I think one ought to use very powerful antimicrobial therapy in the treatment of meningitis.

So far as our own results are concerned, we're doing better. We've been doing better, first, since we handled streptomycin better; second, since we started using,—perhaps, since we started PAS (I have no direct evidence of that); and we are definitely doing better since we've used isoniazid. In fact, isoniazid, in my opinion, has done away with the need for the use of intrathecal streptomycin, a need which Carl Tempel believed wasn't necessary even before that time. We're doing better but we're still going to have some deaths. I do think that isoniazid has smoothed out what I believe was the difference between the fellow with a focus in a bone and meningitis and the fellow with acute generalized miliary meningitis, one which in the streptomycin days, I believe, had a very much worse prognosis than the other.

DR. BUNN: That's a very nice review, Dr. McDermott, and I think all of us are totally

agreed that in a complication of tuberculosis as fatal as is meningitis, shot gun therapy literally is not only indicated; it's imperative. I agree that we will never reduce the mortality rate to zero; we don't even do that in pneumococcal pneumonia. At the same time I am also convinced that we can do far better with the agents at hand than we have done in the past five years, perhaps this year being the change. In other words, I would emphasize that the first question which I threw to the panel is answered favorably. We aren't stationary any longer and perhaps isoniazid is the answer for that; and with the addition of streptomycin with or without PAS, it has made the difference between a standard 60 to 75 per cent mortality to bring it down to perhaps 45 or 50 per cent mortality in the adult. Dr. Lincoln is not sure but she feels that she can improve the survival figures in the children as well. And how far she can state, I'm not sure. Do you think you're going to get 75 or 80 per cent survival, Dr. Lincoln? Do you anticipate it?

DR. LINCOLN: Yes. I think we've been running along on streptomycin-Promizole about 65 per cent, and it looks as if it will be much better with isoniazid.

DR. BUNN: Do you really believe that you need Promizole?

DR. LINCOLN: I think we need a second drug and I think, in children at least, Promizole is just as valuable a drug as PAS. We know that it will cure miliary tuberculosis without the addition of any other drug. We know that it's definitely tuberculostatic; it can be given easily and effectively over a long period of time. We have proof, in a few cases at least, that long administration of it alone does not make the organisms resistant to it, and we also have proof in recent cases, with isoniazid certainly, that it will apparently delay the emergence of resistance as well as PAS. It is much easier to administer if you're going to give it for two years after meningitis, and I would hesitate to try to give PAS for two years to a child at home. I think the difference between the child and the adult may be that Promizole is more effective in the immature individuals, as it was in guinea pigs. Certainly it is a goitrogenic drug and it has certain evidences such as stimulation of secondary sex characteristics that make it possible that it's a more valuable drug in children than it is in adults.

DR. BUNN: I think, without prolonging the arguments about the sulfones, we have other things that are perhaps as important, even more important. During the period immediately prior to this meeting, the panel universally decided that there was no place for the intrathecal administration of streptomycin in the treatment of tuberculous meningitis.

DR. MCDERMOTT: I wasn't there.

DR. BUNN: You've already made the statement on your feet.

DR. MCDERMOTT: No I haven't. I still think there's a place.

DR. BUNN: Like Dr. McDermott, you sometimes have to teach by exaggeration to get the other fellow to talk.

DR. MCDERMOTT: Do you want me to talk to the point?

DR. BUNN: I must admit that the panel arrived at certain of these reasons about intrathecal streptomycin in a variety of ways. Some of them by good reasoning; some of them, perhaps not so good. But perhaps, I think, if there is a difference of opinion we ought to, if we do nothing else, get this problem solved. Is there any time that streptomycin intrathecally should be used? And the Chair, if he has to, will stand high on his feet and yell that intrathecal administration of any drug is medieval.

I think we're trying to get a program of a regimen outlined here at the moment. We're using two drugs and perhaps three, and now we've got to get the route of administration defined.

DR. WALCHER: Paul, I have one comment I'd like to throw in here. We did the best we

could to determine the length of time it took us to get a satisfactory level in spinal fluid of streptomycin by the intramuscular route alone. We, too, have not used intrathecal streptomycin since early in 1949 in our series. I had hoped that we might be able to show by our results that intrathecal therapy was certainly not indicated; but, as I pointed out, because of the extreme lateness with which we received our cases, we had deaths on each side of the fence all the time and I couldn't show it statistically; but we did try to go at it this way by showing the levels of streptomycin which we achieved in the spinal fluid, and we found that we could reach a 5 γ per ml. level within twenty-four to forty-eight hours after the patient had started on his intramuscular streptomycin; and some time thereafter we frequently achieved the level of 10 γ per ml. of spinal fluid. Now, all of the organisms which we believe are going to respond to streptomycin will respond to levels which are below that, so we believe that we have achieved a level in the spinal fluid early in the course of the disease and that is one thing which I think is against the intrathecal administration.

DR. BUNN: Well, obviously Dr. McDermott sits on the other side of the fence, as he often does.

DR. MCDERMOTT: I find myself with a cause, Mr. Chairman.

DR. BUNN: It's a damn long and hard needle which hurts.

DR. MCDERMOTT: First, in reference to Dr. Walcher's remarks, I would certainly agree with them; but I would also point out that we don't honestly know the concentration of drug which is effective against tubercle bacilli. For example, if they are situated intracellularly, 25 to 50 γ per ml. may be necessary to affect those cells. That is, you are comparing the condition in a test tube to a situation in the body where we know that certain portions of the bugs are protected, have a sanctuary within monocytes.

Now, in so far as intrathecal administration of streptomycin is concerned, I don't honestly think there is any great difference of opinion between us, Dr. Bunn. I quite agree with you that it is medieval; it is one of the most sadistic procedures over the long pull that I've seen on a hospital service. But I think a great deal of damage has been done by reasoning by analogy from the Dowling regimens with pneumococcal meningitis to the situation in tuberculosis meningitis. Remember that, before Dr. Dowling introduced the use of a million units of penicillin every two hours by the intramuscular route, a number of patients had been treated for pneumococcal pneumonia, with, we'll say, 50 or 100,000 units of penicillin every two hours and developed pneumococcal meningitis on that regimen. The thing that made the Dowling regimen possible was the lack of toxicity of penicillin, so that one could give virtually any dosage by the intramuscular route. With streptomycin the ceiling on what one can give by the intramuscular route is very obviously low indeed and is probably in the neighborhood of some 3 grams. You can give about that amount intramuscularly and can't give any more and, when you get past that, why, if you're using streptomycin alone, you then have to go to intrathecal therapy. Now Dr. Lincoln has brought in the sulfones, Carl Tempel has brought in PAS and other drugs, and by the use of the other drugs it may well be that one can do away with the intrathecal route, particularly with isoniazid which is distributed so beautifully in the central nervous system. Finally, I'd like to point out that the use of the second drug or the third drug in this situation is not really the same problem as the use of the second drug or the third drug in the treatment of pulmonary tuberculosis, or at least it might not be. In the one case we're talking about the prevention of the emergence of resistant organisms. In tuberculous meningitis, the emergence of resistant infection, although it does occur, is a very rare bird indeed.

DR. BUNN: I think the point is well taken, Dr. McDermott. One has to assume that, if the intrathecal administration of streptomycin is to be avoided, it automatically means

that isoniazid has to be added to the program, primarily because isoniazid with its pharmacologic properties gets into the central nervous system tissues in reasonable concentration and, more important perhaps, from the work of Mackaness and others, it also penetrates the cell wall and acts indeed intracellularly, a phenomenon of which I am aware which is most encouraging.

DR. MCDERMOTT: And it prevents meningitis in patients with miliary tuberculosis.

DR. BUNN: I might get that off the board right now. Has any one seen a patient with tuberculosis being treated with isoniazed develop a central nervous system complication? It is the consensus, I think, of the vast majority that it never occurs. Now, is that true?

At this point several members of the audience described cases with neurological symptoms, none of which were clearly cases of tuberculous meningitis which had developed while isoniazid therapy was being given but instead apparently represented toxic reactions to isoniazid and iproniazid.

DR. BUNN: I think this has got to be very clear. I'm talking about tuberculous meningitis as an infection. The untoward side effects of isoniazid have been reasonably well described, and that convulsions happen, particularly in the susceptible host, is reasonably well documented now. That is different from what I am trying to make a point of, actual spread of an infection during isoniazid. As far as I can make out from conversations in the corridor and on the platform yesterday and here this morning, there has been no dissemination or relapse of a local lesion in the central nervous system for as long as the patient is receiving isoniazid. I think that statement—I would like to have it challenged, but apparently it is not going to be.

DR. MUSCHENHEIM: I don't think this problem of the intrathecal administration of streptomycin has been completely settled as yet. I should like to ask those members of the panel, who felt that, even prior to the introduction of isoniazid, intrathecal treatment was not necessary and not useful, how they reconcile the fact that patients with miliary tuberculosis did develop meningitis but did improve when intrathecal treatment was added. They might not get well but they did improve, and I, for one, am not willing to accept the thesis that, even with isoniazid, intrathecal treatment is never to be used.

DR. MCDERMOTT: I'm not either.

DR. MUSCHENHEIM: In a patient going bad there is still a place for intrathecal treatment, as Dr. McDermott implied.

DR. MCDERMOTT: On a special basis. You would agree that you don't have to do it in every case.

DR. MUSCHENHEIM: I think generally you can get by without it, but I don't think you should rule out intrathecal treatment altogether.

DR. BUNN: I think we would agree to that, Carl. One never makes rigid rules of treatment in any infection as serious as meningitis, pneumococcal or any other variety. I think that would be as a philosophy extraordinarily improper. I didn't mean it in that sense. I was talking about the hypothetical case, the average. Initially I had asked the panel to answer a couple of questions and we have such a verbal group up here that we are way behind time and I'd still like to answer this question from the floor on this problem of relapses, and what about this second question on what is the difference between pediatric tuberculosis in the head and adult tuberculosis. I think Oscar, who has prepared some of this, ought to be allowed the chance to spend two or three minutes talking about both of those problems which I think are very essential in the over-all intelligent management in the long term.

DR. AUERBACH: I don't think there is any question about the fact that tuberculous

meningitis is the result of a hematogenous dissemination. I think the essential difference between the child and the adult is the way in which that dissemination occurs in the brain. Now, because of the frequent association seen up to now, when Paul just dissociated the two, miliary has always been considered with meningitis. As a matter of fact, in most autopsy series in adults, somewhere in the vicinity of 80 to 85 per cent of tuberculous meningitis is associated with miliary tuberculosis. In the child, tuberculous meningitis is by and large the result of a hematogenous dissemination from a primary complex and the primary complex heals, the acute generalized tuberculosis heals, and the tuberculous meningitis results from the rupture of a tuberculous process into the meninges. I don't mean to didactically put that into one, two, and three; but those who have followed carefully the work of Rich and McCordock do believe it is a tuberculous focus which ruptures into the meninges. Well, if your tuberculous primary complex heals and your miliary tuberculosis heals, then the source of dissemination is cut off, and, therefore, we would expect many less relapses in children than we would in adults. In adults, on the other hand, tuberculous meningitis is associated with a high degree of frequency, 75 per cent in adults with active extrapulmonary tuberculosis, chiefly bone or urogenital tuberculosis. When these tuberculous foci develop and progress, there is an involvement of the regional lymph nodes and through that into the blood stream, and a hematogenous dissemination occurs. But the hematogenous dissemination occurs as the result of an active extrapulmonary tuberculosis and you may treat your tuberculous meningitis and do well with it (I'm not talking now about isonizid, we don't know enough about it from an anatomic point of view); you may cure your tuberculous meningitis, your first episode, but you haven't cured your original active extrapulmonary focus. So it isn't unusual for us with adult patients with tuberculous meningitis to see a healing process in one part of the brain and to see a fresh ruptured tuberculous focus into the meninges with a more recent tuberculous meningitis in these areas. I think the difference lies in the pathogenesis that you get a higher cure rate in children than you do in adults.

Dr. Bunn: Dr. Lincoln, you have some ideas on that. Would you speak to the point?

Dr. Lincoln: Well, I believe with Dr. Auerbach that there are many less relapses in children, and I think it is for the reason that he has stated. Therefore, I do think we have more total survivors in children, ultimate survivors, because there are less relapses. But I do not think there is any essential difference between children and adults in the primary treatment. After all, they all died of it before we had any chemotherapy at all. It is true that small infants have a much higher death rate than older children, but, except for that, I don't think there is any difference at all between the primary treatment of meningitis in children and in adults, or that there should be any difference in results.

Dr. Bunn: And one final point which I think needs discussion—that we have other treatment measures for meningitis, and I speak particularly about the proteolytic enzymes used primarily to unblock blocks. For, if the cerebrospinal fluid does not circulate in a proper manner, serious complicating events, in a mechanical sense, can go on in the head. For that reason, even though we are no longer concerned with the intrathecal administration of antituberculous drugs, there seems to be still a place for an agent designed to dissolve certain of the mechanical barriers laid down by tuberculous inflammation. I've asked Dr. Walcher if he would review this, if he can do it in about a minute and a half.—Dwain, I think it would be fine if you could tell us about where and when one should use trypsin or streptokinase-streptodornase—well perhaps even tuberculin—all designed primarily to do the same thing; but the most experience being, I suppose, with streptokinase. Would you like to make a comment on it at all, Dwain, or would you think it is not important enough and we can drop it.

DR. WALCHER: Will you allow me to make just two or three very brief comments? Dr. Honor Smith, as you know, has been the main person to have used intrathecal tuberculin, and I understand she's still doing it, but it has not been widely accepted as a method of therapy in this country, although there are a few single case reports. I do not know of anyone who has used trypsin intrathecally in the treatment of tuberculous meningitis. I should tell you that our professor of biochemistry in Indiana has recently developed a modified trypsin which is considerably different from the trypsin that is commercially available to us now. This trypsin, which has been treated with a hypochlorite, is significantly different in its capacity, or lack of capacity, to produce shock and it also is found to be far more proteolytic. This has been used in many paraplegic dogs; it has been found to have a tremendous influence on the recovery rate both clinically and histologically. I do not know of its use yet in tuberculous meningitis but conceivably that might have some benefit. I should tell you that the ordinary trypsin, the enzyme, has not been shown to have any effect on these paraplegic dogs. Our own experience has been with the use of streptokinase-streptodornase. I should tell you that we have used it now in 16 proved cases of tuberculous meningitis. The results are discouraging. I shall not go into all the elaborate details of our laboratory studies. We had thought at one period of time that it might well be due to the fact that in tuberculous meningitis there was an absence of the plasminogen which is assumed to be a necessary precursor to this reaction. Taking plasminogen levels on many cases of tuberculous meningitis and taking them serially now, we find that that level is no different from that which we observe in the purulent meningitides. It is my own impression that I have seen good therapeutic results from streptokinase in pneumococcal and H. influenzal type B meningitis. So apparently our lack of success is not going to be due to the low plasminogen levels, and we are no further along in our thinking or answering of the problem.

DR. BUNN: Well, Carl, you, this morning at breakfast, said that you would never want to put that stuff in anybody's spinal canal. You want to make a comment about it?

COL. TEMPEL: Yes, I think we lose our meningitis cases from progressive tuberculosis, from resistant organisms or repeated infections, or from these destructive processes. As for blocks and hydrocephalus, we've never saved any of those, and the two patients in which we used streptokinase had febrile responses and a serious increase in the amount of cells. The pleocytosis ruins the fine test you have for learning what progress you are making with the disease. I think it has no place in treatment and I wouldn't use it.

DR. BUNN: The time is up. I would like to spend a couple of minutes to review unless some of the audience would prefer to go on for five minutes to ask some specific questions from the floor. Yes, sir?

From the audience: Of each of the three drugs, how much? how often? how long?

DR. BUNN: I think that is extremely difficult to answer. I was going to put that in the summary about the duration of treatment and dosage scheduling. Actually, if we'll wait for that, I'll try to do it and let the panel argue about it later. Is there another question?

From the audience: We have treated about 250 cases of tuberculous meningitis and we have come to rely rather consistently on the spinal fluid chlorides, diagnostically and prognostically. I have noticed that this test is somewhat conspicuous by its absence from this discussion, and I wondered if you would say something about it.

DR. BUNN: Well, we can review the figures which have been published on it, and I think I can speak to that point, having just reviewed most of the literature. The chloride in all of the past reports of tuberculous meningitis has been used as the single chemical determination of the presence or absence of the infection. And, obviously, if it was low, t has been assumed that the prognosis was worse. In reviewing the Veterans' cases and

certain other series of cases, however, it was very well and clearly shown that approximately one-third of the patients with active proved tuberculous meningitis had normal chloride which remained normal; and, with that significant bulk of patients, I cannot believe that a single chemical determination is going to help in the diagnosis or aid in determining the prognosis of the individual with it. Now, it is in certain laboratories, I realize, still one of the most commonly done procedures, and perhaps it should remain a routine. But as a single test alone without the accessory observations required in the laboratory, I cannot believe that it is a very important one.

From the audience: We do all the other tests with it, and often this test is the decisive one when the sugar happens to be anomalous.

DR. BUNN: Dr. McDermott, would you like to speak?

DR. MCDERMOTT: I just want to make one final comment about something that Dr. Tempel was talking about. That is, just because the host has the ability to react in a certain way does not necessarily mean that that way is of benefit to the total organism. That is the whole basis of psychosomatic medicine (I see Dr. Holmes here with us from Seattle), that is the story in rheumatic fever, and, to a large extent, I believe that is the story in tuberculous meningitis. We start out with what Oscar Auerbach has described as just a few polymorphonuclear leukocytes here and we end up with what, to all intents and purposes, is new tissue capable of caseating and capable of indeed behaving like a tumor; so that there comes a time in tuberculous meningitis when the host's reaction itself becomes the disease. We are helpless to do anything about that with antimicrobial drugs, and must indeed look for better trypsins, better tuberculin therapy, better enzymatic therapies of some other sort. And one final word, I may say I'm distressed to see that at these meetings our methods for evaluating what has happened in the tuberculous patient have so largely centered on microbiologic techniques which I consider of dubious merit, and have taken no part or no account of miliary tuberculosis, Mr. Chairman, which is the one situation in which one can really measure what one is doing.

DR. BUNN: I apologize for eliminating miliary from the discussion first. I agree, though, with what you say, Dr. McDermott, and it is a philosophy which more of us should take into greater consideration. I think, perhaps, that, because of the time and important luncheons and things, if you wish I'd like to summarize what has gone on today and perhaps proceed with the design of a therapeutic program. It might be altered; it might be edited by other people in many ways. First of all, I think the one point which is important is that the disease is reasonably common, that it is a serious and, if not treated early, a completely fatal, disease; that the early diagnosis depends almost solely on the honesty and the integrity of the person investigating both the patient and his spinal fluid; that, if treated early, the patient with meningitis can recover completely and, perhaps for an arbitrary definition, we have three weeks in which to accomplish that. Where a diagnosis is made in the advanced stage, particularly in the stage of coma, that's where probably always the disease is never going to be reversed no matter how powerful the enzymes or the chemotherapeutic agents. One has to assume that the host has diseased tissue which is reversible whether or not the organisms are eradicated from it.

In design for any therapeutic program for a patient with tuberculous meningitis, streptomycin and isoniazid seem to be on an equal footing as basic ingredients. The dosage, it seems to me, can be defined only as a minimum. The patient needs at least a gram of streptomycin seven days a week and isoniazid somewhere between 5 and 10 mg. per kg. of body weight by mouth. And, in those instances where it is not possible or easy to use it orally, there are parenteral preparations. The use of streptokinase and streptodornase, from which we've had our most experience probably, is indicated as one learns what the spinal fluid is doing during the course of treatment. Routine spinal taps should be done

periodically. If the presence of a block is obvious clinically to the person doing the procedure, then the addition of the enzymes probably is indicated. The dosages of those need not be described in detail; they are well described on all of the package inserts.

The other thing which needs definition is duration of treatment. Obviously, from the experience of all of us here, a minimum and a purely arbitrary requirement of 180 days of continuous treatment should be given, no matter what happens to the patient. The continuation of treatment beyond the six-month period probably should be designed as the spinal fluid tells the physician to design it. If the proteins are approaching normal and the cell count is toward normal, and the chlorides and the sugar are back to normal, then perhaps letting up on treatment can be accomplished. But with a disease as apt to relapse and with the difficulty of eradicating the infectious process itself, I think time is reasonably unimportant. Actually treatment should be continued until the patient is well; a minimum of six months, longer in most cases.

It is extremely difficult, I think, to do the most important job here, and that is the old axiom in any infectious disease, that you never eradicate it by treating it. If there were some way in which we could prevent the development of tuberculous meningitis in the patient whose antibodies or humoral or cellular defenses, whatever you wish to call them, are suddenly depleted, to allow the disease to get started in the first place; if there were some answer to that, then the problem of tuberculous meningitis would become reasonably unimportant, no matter which drugs we had available or what gimmicks or gadgets. Until we know more about the host, in other words, and why he develops meningitis or actually a focus any place else, then I don't think we are ever going to eradicate the problem by merely treating, no matter how good its treatment is.

That, in brief, is my final summary. If the panel would have other statements to make on such a program, I would be happy to hear them. This part of it, I must admit was unrehearsed.

DR. SMITH: Dr. Bunn, could I amend your statement or suggest it be amended by increasing the minimum of six months of treatment to twelve months, and further provide that the spinal fluid be perfectly clear for the last six months, as a minimum. Also, I would not want to give up the use of PAS.

DR. BUNN: Dr. McDermott adds one point here which I think is fascinating and we can end on it: Whether or not it might be wise to consider the use of cortisone or perhaps adrenal corticotropic hormone in the artificial effort on the part of the physician to alter basic inflammatory tissue to a point where it becomes acute and potentially capable of being reversed without scar; then, indeed, a study of cortisone and the treatment of tuberculous meningitis might be something to consider for the future.

DR. SMITH: May I say that we have so treated one case, a patient whom we thought was lost and whose recovery to date has been nothing short of miraculous.

DR. TEMPEL: I'd say that our treatment averaged eighteen months so to add to that that a patient should get a year and a half of treatment and in every case of ours this treatment was carried six to eight months beyond the point where normal spinal fluid was found on spinal tap.

DR. BUNN: I think that that other statement is the important one, isn't it Carl? It's not that you want to say that every patient deserves eighteen months of treatment. I don't think you mean that.

DR. TEMPEL: I could just add that PAS is very difficult to give in a very ill patient and it must be given parenterally; it's been our experience to use 15 gm. of lyophilized PAS daily. We used it as long as six weeks in patients who could not take it by mouth. We feel it's an extremely important point in treatment to get either the PAS or isoniazid into the patient parenterally if the patient is in coma or semicomatose and cannot take it orally.

DR. BUNN: End of panel.

NOTES

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY—1954

The American Trudeau Society, medical section of the National Tuberculosis Association, invites the submission of abstracts of scientific papers for presentation at its next annual meeting in Atlantic City, New Jersey, May 17 to 21, 1954.

Full information can be obtained by writing to the Chairman of the Medical Sessions Committee, American Trudeau Society, 1790 Broadway, New York 19, N. Y.

EXHIBITS FOR 1954 ANNUAL MEETING

The Exhibits Subcommittee of the 50th Anniversary Program Planning Committee is well into its initial phase of planning for exhibit presentations at the May, 1954, meeting in Atlantic City.

Heretofore, there have been no commercial exhibits at NTA meetings. However, at the 50th Anniversary Meeting, space for such exhibits is being provided. Selected commercial displays, particularly those which reflect some of the fine work being done in commercial laboratories, will be presented.

Broader areas of interest, including many medical and socio-economic conditions which relate to problems of tuberculosis, will be more adequately represented. Such a broadening of scope will heighten the interest and value of the 1954 exhibits by drawing into them a wider variety of related presentations.

The Exhibits Subcommittee welcomes a wide participation in planning for the 50th Anniversary Meeting.

Anyone interested in sponsoring an exhibit at the meeting should write to Mr. C. Graham Eddy, National Tuberculosis Association, 1790 Broadway, New York 19, N. Y.

POSTGRADUATE COURSE ON DISEASES OF THE CHEST

The American College of Chest Physicians has announced its Sixth Annual Postgraduate Course on Diseases of the Chest to be held in New York City on November 2-6, 1953. Further information can be obtained from the American College of Chest Physicians, 112 East Chestnut Street, Chicago 11, Illinois.

AMERICAN TRUDEAU SOCIETY FELLOWSHIPS IN TUBERCULOSIS RESEARCH

The American Trudeau Society, medical section of the National Tuberculosis Association, provides a limited number of fellowships to promote the training and development of investigators in the field of tuberculosis and related pulmonary diseases. The awards are open to citizens of the United States for work within this country. Preference is given to candidates not more than thirty years of age who have the degree of Doctor of Medicine or other doctor's degree.

Within the broad field of research on tuberculosis, applications will be considered in the special disciplines of clinical medicine and surgery, epidemiology, chemistry, microbiology, pathology, and pulmonary physiology.

Stipends are determined by individual circumstances and may vary from $3,000 to $3,600 per annum. No other remunerative work will be permitted during the tenure of the fellowship. Appointments are for full-time service. They are made for one year, but they may be renewed up to a total period of three years. Fellowship applications are

considered at meetings of the Fellowship Board of the American Trudeau Society in October and April or May; to receive consideration at these meetings they must be received by September 1 or April 1. Appointments may begin on any date determined by the Board.

Further particulars concerning these fellowships may be obtained on request.

Address communications to: The Director of Medical Research, American Trudeau Society, Henry Phipps Institute, Seventh and Lombard Streets, Philadelphia 47, Pennsylvania.

FIFTH URUGUAY CONGRESS ON TUBERCULOSIS

The Fifth Uruguay Congress on Tuberculosis was held in Montevideo on September 24, 25, and 26, 1953, under the sponsorship of the Ministry of Public Health. Subjects discussed were:

Clinical, Anatomopathologic, and Radiologic Aspects of Tuberculosis in the Adult and Child;

Pulmonary Resection for Tuberculosis; and

Characteristics of the Bacillary Infection in the Study of the Tuberculous Environment.

Doctor Armando Sarno was President of the Executive Committee.

TENTH PAN AMERICAN CONGRESS OF THE UNION LATINO AMERICANA DE SOCIEDADAS DE TISIOLOGIA

The American Trudeau Society, medical section of the National Tuberculosis Association, is sending four observers to the Tenth Pan American Congress of the Union Latino Americana de Sociedades de Tisiologia, to be held December 5–10, 1953, in Caracas, Venezuela. These observers will be Dr. Esmond R. Long, Dr. Herbert L. Mantz, Dr. Donald S. King, and Dr. Joseph B. Stocklen.

THE AMERICAN REVIEW OF TUBERCULOSIS

ABSTRACTS

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CLINICAL STUDIES, TUBERCULOSIS

Round Pulmonary Foci.—Anatomic study of resected lung specimens reveals the diversity in the pathologic structure of round tuberculous pulmonary foci. (*1*) Tuberculomas tend to progress at the site of caseation without liquefaction; a stratified lesion is produced by alternating phases of caseation and fibrosis as in the 20 tuberculomas examined. The concentric enlargement can be observed on serial roentgenograms which sometimes reveal the lamellar structure as well. The central nodule around which the successive caseous layers develop most often is a reinfection focus; occasionally, it may be a partly calcified primary lesion or a small developing cavity. Because of their progressive character, such foci reach considerable size. (*2*) Homogeneous caseous foci without stratification are not progressive and arise from massive caseation, which subsequently becomes encapsulated. These lesions, especially when large, develop into cavities with thick caseous walls. If small, such foci often regress. (*3*) Round foci consisting of conglomerate nodules assume a round homogeneous form because of surrounding atelectasis. They are often regressive and are particularly frequent under pneumothorax. (*4*) Round filled-in cavities are rare. (5) Inspissated cavities are pathogenetically and structurally entirely different from tuberculomas, with which they have frequently been confused. In contrast to filled-in cavities, inspissated cavities have a smaller diameter than the original ring-shadow. The outline is more often ovoid than round and somewhat irregular due to retraction. The caseous material is not lamellar.—*Tuberculomes et foyers ronds (essai de démembrement) a propos de 37 cas, dont 33 anatomo-cliniques, Santy, Berard, Galy, & Prignot, Rev. de la tuberc., 1952, 16: 1075.*—(*V. Leites*)

Tuberculous Meningitis and Cerebral Tuberculosis.—Among 44 children and 21 adults who died from tuberculous meningitis in Buffalo institutions, the meningeal process was an extension from the brain to the meninges in only 5 children and one adult. In most cases, a primary leptomeningeal tuberculoma was present. In patients treated with streptomycin, the course of the disease became more chronic and the size of meningeal tuberculomas much larger.—*Meningitis tuberculosa: Observaciones en niños y adultos, con algunas observaciones sobre la patogenia y sobre los efectos del tratamiento estreptomicínico, K. Terplan, Rev. tuberc., Habana, January–June, 1952, 12: 1.*—(*A. A. Moll*)

Primary and Reinfection Tuberculosis in Peru.—A pathologic study included 50 representative autopsies on adult tuberculosis patients who died from the disease. Of these patients, 32 were half-breeds, 10 white, 4 Negro, and 2 were pure Indians. In 9, death was caused by a primary infection and in 28 by a reinfection, while in 13 the morphology of lesions was not sufficiently characteristic to establish the nature of the process. Primary cases were mostly among Indians and persons recently moving to Lima from the country. The comparatively high prevalence (18 per cent) of primary tuberculosis in the series suggests that childhood infection is not invariably a fact in Lima.—*Patología de la infección tuberculosa primaria en el adulto, J. Campos Rey de Castro, Rev. tuberc., Habana, January–December, 1951, 11: 1.*—(*A. A. Moll*)

Chest Wall Tuberculosis.—Most of 62 proved cases of tuberculous abscess or sinuses of the chest wall occurred in adolescents or young adults, and 69 per cent of the patients had evidence of present or past tuberculous

infection elsewhere in the body. Total or subtotal excision of the abscess or sinus tracts, paying particular attention to the portion underneath the ribs, was the procedure of choice. Complete hemostasis and obliteration of the dead space with pedicle muscle graft were emphasized. The results were favorable in 87 per cent of the patients. General antituberculous treatment was important in the preoperative and postoperative periods. Penicillin and streptomycin helped control infection and ensured healing of the wound by primary closure. A minimal period of three months of bed rest following surgery was recommended in every case.—*Tuberculosis of the chest wall and its surgical treatment, Y. K. Wu, T. H. Chang, & H. F. Ting, Chinese M. J., May-June, 1952, 70: 213.*—(*L. Hyde*)

Skeletal Tuberculosis in Brazil.—In twelve orthopedic hospital departments in four Brazilian cities, tuberculosis of the bones or joints was diagnosed in 282 of 10,236 patients under treatment. The rate of 2.7 per cent is too low; when two services specializing in fractures were left out of the computation, the percentage rose to 6.8 per cent. This is closer to the facts and is far above that reported for similar institutions in the United States. The distribution by sites showed Pott's disease, 48 per cent; tuberculous coxitis, 27.6 per cent; tuberculosis of the knee, 18 per cent; and other locations, 5.3 per cent. These figures vary somewhat from those given for Europe and the United States. The problem deserves attention and an inquiry should be made to determine more accurately the number of cases.—*Tuberculose ósteo-articular (Freqüência das formas clínicas; Assistência médico-social no Brasil, G. D. Velloso, Clin. tisiol., May-June, 1952, 7: 327.*—(*A. A. Moll*)

Pott's Disease in Peru.—A study in the Children's Hospital of Lima shows that tuberculous spondylitis in children is the most common type of bone and joint tuberculosis in Peru, one-third of the cases being spondylitis. Among 318 cases of Pott's disease in the hospital, boys were slightly predominant. The most commonly involved sites were the lower thoracic and lumbar areas. Extension to other skeletal areas with multiple bone and joint lesions was found in 11 cases (2.51 per cent). Pulmonary involvement was present in 30.81 per cent of the cases. Streptomycin, at a daily dosage of 0.5 gm. and a minimum total dose of 80 gm., gave gratifying results from the standpoints of increased weight, improved appetite, and closing of sinuses. The death rate was 27.9 per cent, the most common causes of death being meningitis, pulmonary tuberculosis, and peritonitis.—*Mal de Pott infantil, A. Ray Helden, Rev. tuberc., Habana, January-June, 1952, 12: 151.*—(*A. A. Moll*)

Pulmonary Osteo-Arthropathy in Dogs.—Dogs affected with pulmonary tuberculosis or primary pulmonary neoplasm may develop chronic pulmonary osteo-arthropathy infrequently, 10 cases having been reported. The present case occurred in a dog which had fibrosarcoma of the lungs proved at autopsy. The osteo-arthropathy may occur in either sex. It is more likely to be found in large breeds of dog, and in all except one case there were primary lesions in the lungs. It is noted that chronic pulmonary osteo-arthropathy does not appear to be associated with metastatic neoplasms of the lungs.—*Chronic pulmonary osteo-arthropathy in the dog, G. Mather & D. Low, J. Am. Vet. M. A., March, 1953, 122: 167.*—(*L. Hyde*)

Pathogenesis of Post-Excisional Fistulas.—Fistulas after pulmonary excision in tuberculous patients resulted from extension of tuberculous empyema, which preceded the fistula in one-third of the cases. These fistulas occurred later after operation than those in nontuberculous patients. In the latter, fistulas appeared within thirteen days postoperatively and presumably were due to technical errors in stump closure. Neither tuberculous ulcers of the bronchial stump nor hilar lymph node tuberculosis is an important source of bronchopleural fistula. Maier, discussing the paper, stated that more fistulas occurred when inter-

mediate amounts of streptomycin were used in several short courses; the best results followed excisions performed after the first course of streptomycin while the bacilli were still sensitive or after a prolonged uninterrupted course of streptomycin combined with *p*-aminosalicylic acid (PAS). If excision could not be performed with the first course, it was delayed until some months of treatment with streptomycin and PAS had produced maximum subsidence of the tuberculous process.—*The pathogenesis of bronchopleural fistulas following excisional therapy for pulmonary tuberculosis, G. N. Stemmermann, C. F. Daniels, & O. Auerbach, J. Thoracic Surg., October, 1951, 22: 393.—(R. E. MacQuigg)*

Tuberculous Pleural Empyema.—In a series of 22 cases of tuberculous empyema, 20 (91.8 per cent) developed during an ineffective pneumothorax. Treatment depends upon closing the empyema cavity. To prevent further trouble, collapse therapy should be discontinued and Jacobeus operation substituted. If an effusion develops after pneumothorax, frequent aspirations are indicated to dry the pleural space. A bronchopleural sinus which persists after thoracoplasty is best treated by pneumonectomy with total pleurectomy followed by thoracoplasty.—*Contribución al estudio del empiema pleural tuberculoso, V. Narváez Obeso, Rev. tuberc., Habana, January–December, 1951, 11: 99.—(A. A. Moll)*

Apicolysis with Plombage.—Plombage apicolysis may be considered as definitely incorporated into the armamentarium against pulmonary tuberculosis. This applies particularly to bilateral lesions located in the apical area. Because of its mild aftereffects, the procedure may be carried out even in poor surgical risks. In a series of 50 patients of both sexes so treated, the age ranged from fourteen to forty-eight years. In 45 cases, conversion of sputum and clinical and roentgenologic improvement followed. In 5, the sputum remained positive, but the clinical picture improved. In all cases, streptomycin was given preoperatively.—*Apicolisis con plombaje, S. Huaco Vizcardo, Rev. tuberc., Habana, January–December, 1951, 11: 92.—(A. A. Moll)*

Results of Thoracoplasty.—Thoracoplasty is the best surgical collapse procedure for chronic apical tuberculous cavities. The results are inversely proportional to the size of the cavity and directly related to its location. The surgical technique should be governed by the morphology and topography of the lesion. Monaldi's drainage should be used as a supplement for tension cavities and Maurer's method for extensive posterior lesions. In selected cases, the first rib may be retained. The anesthetic agent of choice is ether-oxygen, unless contraindicated. Cotton thread is an adequate suture material. Penicillin and streptomycin both preoperatively and postoperatively should be a routine. Among 461 patients treated in the 1938 to 1950 period, 53.1 per cent were apparently cured, 21.9 per cent improved, 10.5 per cent were unchanged, and 11.0 per cent progressed. There were 3.5 per cent postoperative deaths. Among the latest 200 patients who received antibiotics, the apparently cured rose to 70.5 per cent; the improved dropped to 15.0 per cent; those unchanged dropped to 5 per cent; those progressing dropped to 7 per cent; and those dying immediately dropped to 2.5 per cent.—*A thoracoplastia na colapsoterapia da tuberculose, B. Gontijo, Clín. tisiol., May–June, 1952, 7: 308.—(A. A. Moll)*

Segmental Resection in Pulmonary Tuberculosis.—The series reported includes 6 cases in which pulmonary segmental resection was performed alone for pulmonary tuberculosis and 3 others in which segmental resection and lobectomy were used together. The outcome was favorable, except in 3 cases in which a postoperative sinus and empyema followed.—*Considerações sôbre alguns casos de resecções segmentares no tratamento da tuberculose pulmonar, E. de J. Zerbini, Clín. tisiol., May–June, 1952, 7: 251.—(A. A. Moll)*

Chest Roentgenograms.—Because various

structures have different densities and shadows overlap, it is often difficult to obtain satisfactory roentgenograms of the chest. This is especially true in trying to correlate apical changes in the lungs with the so-called "cervico-thoracic diaphragm" or septum. In such cases, planigraphy is too costly and time-consuming. Hoffstaedt's technique, preferably in a recumbent posture with a 35-degree angulation of the tube, solves this problem.—*Exploración radiológica de los vértices pulmonares, M. Espinosa Gallarza, Rev. tuberc., Habana, January-December, 1951, 11: 86.*—(*A. A. Moll*)

Bronchography and Pulmonary Tuberculosis.—Adequate bronchograms give more information about the condition of the lung than is generally apparent. This information cannot be obtained by such means as routine chest roentgenograms, planograms, or bronchoscopy. Routine chest roentgenograms often do not show exactly which segments are diseased and cannot reveal whether normal anatomy is restored with resolution of the lesion. A careful study of post-bronchographic films over long periods has shown no harmful effects attributable to the iodized oil in cases of pulmonary tuberculosis. When the examination is made with care and minimum quantities of oil are used, the patient can remove practically all of the oil by postural drainage. Patients with progressive disease or excessive secretions may have spread of disease or retention of secretions when the cough reflex is temporarily blocked by topical anesthesia. Simultaneous bronchoscopy and bronchography is less successful than separate procedures except for patients with marked stenosis of a main bronchus. Here, bronchoscopy is done on the roentgenogram table and an aqueous contrast medium is instilled under fluoroscopic control. As soon as films are exposed, the medium and secretions are aspirated. Non-oily solutions are not recommended for routine bronchograms. Bronchograms frequently show structural changes in areas where resolution has occurred and such cases might fare better with permanent collapse. Prethoracoplasty bronchograms might also indicate why some thoracoplasties are unsuccessful. When decortication is being considered, bronchography best shows the structural changes in the collapsed lung and aids in deciding whether the lung is healthy enough to make the procedure advisable.—*Bronchography as an aid in planning surgical treatment of pulmonary tuberculosis, J. Gordon, W. B. Zinn, R. Brook, & P. C. Pratt, J. Thoracic Surg., August, 1951, 22: 109.*—(*R. E. MacQuigg*)

Avian Tuberculosis in Pigs.—Natural infections of pigs with avian tuberculosis have been reported but pig-to-pig infection has been thought to be unusual. To determine whether swine can infect each other, 2 tuberculin-negative pigs were repeatedly fed chicken tissue infected with avian tubercle bacilli. Six weeks after the initial feeding, both animals reacted slightly to avian tuberculin but not to bovine tuberculin. Four months after the initial feeding, 3 small pigs were put in the pen with the 2 original pigs. The 3 contact pigs did not react to avian tuberculin after three months' exposure, and a month later no avian bacilli could be found in the feces of the original animals. All 5 pigs reacted to avian tuberculin six months after the 3 young animals were placed with the 2 originally infected. A month later, 4 of the pigs were autopsied; the 2 original and one contact pig had calcified lesions in the mesenteric lymph glands, the remaining contact animal had submaxillary lesions. All of these foci yielded avian tubercle bacilli. It thus seems that there was pig-to-pig spread of avian tuberculosis.—*Avian tuberculosis infection in swine, R. Gwatkin & C. A. Mitchell, Canad. J. Comp. Med. & Vet. Sc., October, 1952, 16: 345.*—(*L. B. Hobson*)

CLINICAL STUDIES, NONTUBERCULOUS DISEASES

Constrictive Pericarditis.—Since 1937, pericardectomy has been performed on 45 patients, 25 men and 20 women. They were 11 to 58 years old, the average age being 29; all of

those showing calcification were older than 20 years. Although it was often impossible to date the onset of pericarditis, at least two years seemed necessary for calcification to appear. The development of fibrosis sometimes was acute, in one case appearing in two months. The lesion was proved to be tuberculous in 18 others (71 per cent in all). Two cases were post-traumatic and one rheumatic. Of the 10 cases of unknown etiology, 9 showed calcification; 3 of these gave a negative tuberculin reaction. Of the 14 proved tuberculous cases, 9 showed calcification. Symphysis never followed purulent pericarditis. Of the initial symptoms, digestive troubles were the most common, probably secondary to hepatic congestion. Hepatomegaly occurred in 43 patients and ascites in 38. The rapid reaccumulation of fluid after removal was a valuable point in differentiating pericarditis from tuberculous peritonitis. Dependent edema was noted in 29 cases and hydrothorax in 19. Cyanosis was present in 33 patients but was never severe; dyspnea was a nearly constant finding. Venous pressure was consistently elevated and the circulation time was augmented. Cardiac signs were not prominent, although half of the patients had a protodiastolic murmur, usually typical of a pericardial vibration but occasionally resembling that of mitral stenosis. Electrocardiographic changes included low voltage and inversion of T waves. Radiologic signs were particularly helpful if anteroposterior and lateral tomography were included. The latter was useful in localizing calcification preoperatively. In the absence of calcification, the diagnosis was sometimes obscured by a fibrous band around the auricle or even the left ventricle, producing a "pseudo-mitral" aspect. In the old fibrocalcific cases, localized rigidity was frequently noted on fluoroscopy but this sign was often lacking in the presence of fibrotic bands. Often pleural effusion masked the cardiac contour. There were 4 clinical forms: (*1*) pseudo-cirrhotic and pseudotuberculous peritonitis, (*2*) that characterized by infantalism (5 cases), (*3*) an almost latent form (3 cases), and (*4*) an acute form (17 cases) in which symptoms appeared in one to eleven months following acute pericarditis. The operative indications were based on the clinical course. In the acute cases, operation was urgent. Streptomycin revolutionized the treatment by removing the fear of early intervention when the disease was progressing rapidly toward constriction. In the chronic forms, operation was not urgent but nothing was gained by delay. The problem was a more delicate one in patients with minimal signs and in these decision had to be based upon such factors as age, occupation, and psychological make-up. Of the 45 patients operated upon, 8 died postoperatively (18 per cent) and 3 died later. Among the 34 survivors, 24 were completely cured and led normal lives. The others had fair to mediocre results. Before 1946, the operative mortality was 26.6 per cent; after 1946, it dropped to 13.3 per cent. The percentage of cures was also better in the later group. There was no significant difference in results between the calcified and fibrotic symphyses. Operative deaths were fewer, and good results were more frequent in the chronic than in the acute forms. The use of streptomycin may explain the increasing incidence of constrictive pericarditis in that more patients survive the acute stage.—*Traitement chirurgical des symphyses du péricarde, P. Santy, P. Marion, & J. Gravier, Poumon, June-July, 1952, 8: 507.*—(*F. S. Lansdown*)

Constrictive Pericarditis.—Between early 1949 and the end of 1951, 17 patients were operated upon for constrictive pericarditis. Of these 17, only 9 had evidence of tuberculosis and one of rheumatic fever. The etiology was unknown in the others. The calcified forms should be distinguished from the fibrotic forms, although all probably begin as fibrosis and, after a period of latency, may calcify. The prognosis in the calcified cases is a great deal better; the disease is unquestionably inactive and a partial pericardectomy usually disembarrasses the heart. There is real danger of reactivation in the fibrotic forms; operation is often urgent, the patient is in poor general

condition, and a total pericardectomy is usually necessary. In these cases it is preferable to operate from a left approach and remove as much pericardium as is feasible. Later, after the patient has benefited from the improved circulation, the pericardectomy is completed from the right. Among the patients in the calcified group, 9 per cent died, 36.5 per cent improved, and 54.5 per cent were cured. Among those in the fibrotic group, 35.3 per cent died (23.4 per cent immediately and 11.9 per cent later), 23.5 per cent improved, and 41.2 per cent were cured.—*Les péricardites constrictives et leur traitement chirurgical; Résultats opératoires éloignés, F. D'Allaines, C. Dubost, & R. Verost-Veroft, Poumon, June-July, 1952, 8: 541.*—(*F. S. Lansdown*)

Constrictive Pericarditis.—Among 20 cases of pericarditis operated upon between June, 1947, and May, 1951, tuberculosis was proved in 18; 17 were constrictive pericarditis and 3 acute. The acute cases did not respond to conservative therapy and operation was undertaken as a last resort in gravely ill patients. Among the older cases, tubercles and tuberculous granulation tissue were frequent without any clinical signs of activity. From one to twenty-four months elapsed between the acute infection and the development of signs of constriction. The most important diagnostic signs were cyanosis, increased venous pressure, enlarged liver, ascites, and hydrothorax. The local signs most often encountered were protodiastolic murmur, T wave changes in the electrocardiogram, and an enlarged heart shadow with barely perceptible pulsations. Patients were prepared preoperatively with strophanthine, mercurial diuretics, and paracentesis when necessary. Streptomycin was used in full dosage in only 2 instances. The optimal moment for intervention was when the active process had subsided and before irreversible myocardial or liver changes had occurred. The exact time was variable and there are no dependable criteria to determine it; in general, operation was more effective if performed early, even if the infection were still active. At operation, an approach from the left and then the right was made, and as much pericardium as possible was removed. No attempt was made to free the entrance of the venae cavae. Among the 17 patients with constrictive pericarditis, 9 were cured, 2 were much improved, 2 remained stationary, and 4 died (2 of these early and 2 late). Among the 3 with acute tuberculous pericarditis, one was cured, one remained stationary, and one died.—*Contribution a l'étude clinique et thérapeutique des péricardites chirurgicales, V. Stoyanovitch, Poumon, June-July, 1952, 8: 547.*—(*F. S. Lansdown*)

Constrictive Pericarditis.—The etiology of most cases of constrictive pericarditis is not well understood. Tuberculosis has been implicated in 10 to 28 per cent of the cases in various series. The remainder have been ascribed to acute pyogenic infections, rheumatic fever, polyserositis, or unknown causes. The relationship between traumatic pericardial hemorrhage and later chronic constrictive pericarditis has perhaps been overlooked. Chronic pericardial effusion and pericardial hemorrhage may remain relatively asymptomatic for the long periods before symptoms of constrictive pericarditis become apparent. This latent period of varied length has been mentioned in the few reported cases. Another patient presented additional evidence for this etiology. Experiments in dogs indicated that constrictive pericarditis may result from intrapericardial hemorrhage and suggested that the lipid fraction of blood is probably the most significant component in its production. Similar experiments should be carried out, using repeated injections of blood and lipid fractions to stimulate more long-standing hemopericardium.—*Hemopericardium and constrictive pericarditis, J. L. Ehrenhaft & R. E. Taber, J. Thoracic Surg., October, 1952, 24: 355.*—(*R. E. MacQuigg*)

Right Pericardial Fat Pad.—In some obese patients, a pericardial fat pad is present at the level of the right auricle and superior vena cava. It can produce a prominence of

the upper half of the right border of the cardiovascular shadow as seen on a postero-anterior roentgenogram of the chest as in 2 recent cases which were autopsied. The knowledge that this structure is occasionally present may aid in explaining a prominence of the upper part of the cardiovascular shadow which otherwise might be interpreted as a dilated superior vena cava.—*The right pericardial fat pad, S. L. Cohen, Radiology, March, 1953, 60: 391.*—(*W. J. Steininger*)

Bronchial Erosion.—During a period of fifteen years, 48 patients have been observed for whom the history, specialized examinations, and subsequent observations have seemed to justify a diagnosis of simple erosion of the wall of a small bronchus with pulmonary bleeding. Some patients had colds shortly before the bleeding began, while other ones engaged in fairly strenuous physical exertion within a day preceding the bleeding. There were 35 men and 13 women in this group, aged from 17 to 65 years. All patients were examined and treated bronchoscopically at the time of active bleeding, which usually occurred from the posterior or posterolateral division of the bronchus to the lower lobe. Treatment consisted of dilatation of the bronchus, mechanical curettement of the bronchial wall with an aspirating tube, instillation of a 1 per cent solution of phenylephrine hydrochloride or topical thrombin, and, finally, insufflation of a mixture of sulfanilamide and sulfathiazole powder into the affected bronchus.—*Simple bronchial erosion as cause of pulmonary hemorrhage, P. P. Vinson, J.A.M.A., November 1, 1952, 150: 840.*—(*H. Abeles*)

Idiopathic Hemoptysis.—A study was made of the case records of 75 patients who had hemoptysis for which no cause could be detected. All patients had roentgenographic, bronchoscopic, and bronchographic examinations. Follow-up information five or more years later was obtained in 55 patients. Of these patients, 50 were alive five or more years after the date on which the diagnosis of idiopathic hemoptysis was made. Of the 8 known deaths in the entire series, 3 are known to have been due to primary pulmonary disease, one of them due to bronchogenic carcinoma and the other 2 probably due to bronchiectasis. The prognosis in idiopathic hemoptysis is good, particularly in cases in which the hemoptysis does not become a recurrent symptom.—*Prognosis in idiopathic hemoptysis, B. E. Douglass & D. T. Carr, J.A.M.A., October 25, 1952, 150: 764.*—(*H. Abeles*)

Bronchiectasis.—In 75 patients with severe bronchial asthma, bronchography was done without ill effects. Bronchiectatic changes were demonstrated in 27 patients, of whom 13 had the disease bilaterally. Exploratory operation was done on the 27 bronchiectatic patients, all but one of whom were treated by excision of the diseased tissue, 4 patients having bilateral resection. Two patients suffered infectious complications postoperatively and were not improved. Of the 26 patients treated surgically, 20 are greatly improved in health and are able to work; only 7 require bronchodilator drugs. Bronchiectasis is often present in the asthmatic patient. Removal of the abnormal segment can bring permanent relief.—*Hidden or unsuspected bronchiectasis in the asthmatic patient, R. H. Overholt, J. H. Walker, & F. M. Woods, J.A.M.A., October 4, 1952, 150: 438.*—(*H. Abeles*)

Argyrophil Epithelial Barrier in Bronchiectasia.—In 11 surgical specimens from bronchiectasic cases, del Rio Hortega's silver carbonate stain was used to demonstrate the persistence of Polak's so-called "epithelial barrier" in the bronchial lining. The barrier was continuous except when severe inflammatory processes were present.—*Nota sôbre a "barreira epitelial" argentófila na bronquiectasia, D. de Paola, Rev. brasil. tuberc., September–October, 1952, 20: 577.*—(*A. A. Moll*)

Pulmonary Amyloidosis.—A 39-year-old white man with a history of recurrent pneumonias, pleurisy with effusion, persistent

tenacious mucoid sputum, occasional hemorrhage, hoarseness, and wheezing died from progressive respiratory failure. At autopsy, amyloid material was found in the larger radicals of the bronchial tree, in the bronchioles, and in some of the alveolar septa. It was chiefly in the submucous connective tissue in close relation to the cartilagenous substance. There were also deposits in the subintimal connective tissue of branches of the pulmonary artery. The tongue was free of amyloid as were the other organs.—*Diffuse amyloidosis in the lower air passages, O. Noring & H. Paaby, Acta path. et microbiol. Scandinav., 1952, 31: 470.*—(*H. G. Turner*)

Silicosis.—Post-mortem and postoperative studies reveal certain pulmonary lesions which are not recorded on conventional roentgenograms. Some of these lesions are more than 1 cm. in diameter. Isolated conglomerate or small massive silicotic lesions are sometimes called carcinoma of the lung, especially in those instances in which one does not see roentgenographic evidence of nodulation. Migration of massive silicotic lesions of the upper lobes toward the hilum has been demonstrated in certain patients who were followed for ten or more years. The roentgenographic appearance of a silicotic lesion which has migrated toward the hilum is frequently that of a mediastinal tumor (Author's summary).—*Some problems in roentgen diagnosis of silicosis, E. P. Pendergrass, J.A.M.A., November 22, 1952, 150: 1178.*—(*H. Abeles*)

Respiratory Manifestations of Typhus Fever.—In a series of 20 cases of typhus fever at La Oroya, Peru, 8 (40 per cent) showed respiratory changes, usually pneumonitis. Chloromycetin controlled the systemic infection and brought about regression of pulmonary manifestations.—*Alteraciones pulmonares en el tifus exantemático, C. A. López García, Rev. tuberc., Habana, January-June, 1952, 12: 152.*—(*A. A. Moll*)

Histoplasmotic Pulmonary Calcification.—A recent case emphasized the need for considering histoplasmosis when the roentgenogram suggests pulmonary tuberculosis but the tuberculin test and other routine examinations are negative. The histoplasmin test may clarify such cases; it was positive in this instance.—*Infiltración pulmonar con sensibilidad a la histoplasmina, A. Pareja Coronel, Clín. tisiol., May-June, 1952, 7: 263.*—(*A. A. Moll*)

Pulmonary Lesions of Brazilian Paracoccidioidomycosis.—A series of 10 patients with pulmonary forms of South American blastomycosis were studied clinically and roentgenologically. The results of the paracoccidioidin test had only presumptive diagnostic value. On the basis of roentgenographic data, the pulmonary lesions were classified as predominantly nodular, predominantly fibrous, or predominantly infiltrative. Tomography was disappointing in this series and did not reveal clearly the nodules and cavities. The most effective therapeutic agents were sulfonamides, which brought about healing through fibrous transformation of the lesions. Cortisone was tried empirically in 5 cases, with somewhat encouraging results; its final place in therapy must await further studies.—*Considerações em tôrno das localizações pulmonares da paracoccidioidose brasileira, J. Machado Filho & M. da C. Mendonça de Carvalho, Rev. brasil. tuberc., September-October, 1952, 20: 503.*—(*A. A. Moll*)

Eosinophilic Pneumonopathy.—A case was characterized by asthmatic bronchitis, eosinophilic leukocytosis, and pulmonary infiltration. Since the features of this illness resembled Löffler's syndrome, tropical eosinophilia, and bronchial asthma, the term "eosinophilic pneumonopathy syndrome" has been employed. This illness was effectively treated with neoarsphenamine (Author's summary).—*Arsenotherapy in the treatment of eosinophilic pneumonopathy, W. G. Ensign, J.A.M.A., November 22, 1952, 150: 1205.*—(*H. Abeles*)

Bronchial Asthma.—A new technique used

in making roentgenograms of the chest in asthmatic patients gives considerable information regarding the pulmonary function of the patient. The first roentgenogram is taken during forced inspiration. Immediately after, a second roentgenogram is taken in deep expiration. The roentgenograms are then superimposed one on the other and studied comparatively. The changes of the diaphragm, ribs, and clavicles occurring during expiration are sketched on the roentgenogram which was taken during forced inspiration. The mechanism of respiration and the participation of each factor involved can thus be determined. —*New roentgenographic technique in bronchial asthma, C. J. Daz, C. Albert, V. L. Barrantes, F. Lahoz, L. Salgado, & C. Lahoz, J.A.M.A., November 29, 1952, 150: 1297.*—(*H. Abeles*)

Localized Pulmonary Hypertrophic Emphysema.—An infant made a good recovery after lobectomy for massive hypertrophic emphysema of the middle lobe of the right lung. All of the 9 well-documented cases of the disease thus far reported occurred in infancy or early childhood. Among these, 7 were subjected to lobectomy or segmental resection without mortality. The disease seems to be a clinical entity of congenital origin, and resection should be done promptly when the diagnosis is made. Shaw reported unusually flaccid bronchi in resected specimens and suggested that a localized bronchial chondromalacia might be the underlying pathology.—*Localized pulmonary hypertrophic emphysema, M. H. Williams, J. Thoracic Surg., November, 1952, 24: 522.*—(*R. E. MacQuigg*)

Surgery for Pulmonary Emphysema.—Advanced pulmonary emphysema was treated in 11 patients by removing degenerative and bullous disease from one lung, completely denervating the pulmonary plexices, and by parietal pleurectomy. Complete vagal denervation was accomplished by severing all of the hilar branches including those from the left recurrent laryngeal nerve, and dissecting the perivascular and peribronchial tissues with the inferior pulmonary ligament. The sympathetic chain was removed from the third through the ninth ganglion, including the splanchnic nerves. The patients tolerated the surgery well. One death occurred thirteen days postoperatively due to persistent air leaks. In the discussion, Carter stated that these chronic invalids improve temporarily with almost any treatment if it is done carefully. If they are hospitalized and their bronchial drainage is increased with ammonium chloride, if they are given oxygen with a pressure apparatus, if they are given enough antibiotics, they improve. If pneumoperitoneum functions effectively and is well tolerated, it may be abandoned after six months; the patient will maintain some of his improvement for as long as two years.—*Surgical management of pulmonary emphysema, G. L. Crenshaw & D. F. Rowles, J. Thoracic Surg., October, 1952, 24: 398.*—(*R. E. MacQuigg*)

Bronchogenic Cyst.—A case of bronchogenic cyst was associated with other congenital anomalies, including the Klippel-Feil syndrome.—*Mediastinal bronchogenic cyst and Klippel Feil syndrome: Report of a case, A. N. Bracher & A. R. Koontz, J.A.M.A., November 8, 1952, 150: 1006.*—(*H. Abeles*)

Multiple Origin of Bronchogenic Carcinoma.—Although the unicellular or unicentric origin of neoplasm has been disputed, only Lindberg's meticulous study has shown that multiple origins are more than sporadic occurrences in pulmonary neoplasms. To study this problem, large tissue sections were prepared from 87 lungs containing bronchogenic carcinoma. In 5 of these, there were 2 grossly visible primary carcinomas in the same or different lobes. In 25 other specimens, there were microscopic plaques of pre-invasive or early invasive carcinoma near the main tumor or far enough from it to be considered independent lesions. In 53 per cent of the cases, the major tumor contained 2 to 5 different histologic patterns arranged in such a manner as to imply initial independence and ultimate

fusion of neighboring neoplasms. Of the 87 specimens, 63 exhibited one or more of these features. These observations support the hypothesis that bronchogenic carcinoma may often arise from more than one site and that clinical recognition frequently awaits the coalescence of independent growths. In commenting on this paper, McDonald pointed out that between 15 and 20 per cent of the squamous-cell carcinomas of the bronchus are noninvasive proximally. Such lesions cannot be recognized grossly and are important to the thoracic surgeon because they recur in the stump if he cuts across them. In one case reported by Papanicolaou, the entire squamous lesion was *in situ* in the bronchus; the positive cytologic examination would have been considered false had not the lesion been resected and the patient unquestionably cured.—*Bronchiogenic carcinoma, a product of multiple sites of origin, E. J. McGrath, E. A. Gall, & D. P. Kessler, J. Thoracic Surg., September, 1952, 24: 271.*—(*R. E. MacQuigg*)

Adenocarcinoma Within Bronchial Adenoma.—Although the malignant potentialities of bronchial adenomas are no longer doubted, well-authenticated cases of bronchial adenoma showing clear-cut histologic evidence of transition to adenocarcinoma have not been reported. In one case, a routine chest roentgenogram showed an oval density in the right lung peripherally. It suggested pulmonary tuberculosis but there was no bacteriologic confirmation. Six years after its discovery, the lesion suddenly enlarged and cavitated, which was interpreted as extension of a tuberculous process with central necrosis. Right upper lobectomy was performed. The tumor appeared to arise from multiple foci in the bronchial mucosa. Foci of squamous metaplasia to apparent malignancy were numerous in the bronchi within the tumor. Proved adenocarcinomatous change in a bronchial adenoma further emphasizes the necessity of treating adenomas by pulmonary resection. Such cases as this challenge the policy of "watching" round lesions which are thought to be tuberculous in nature.—*Adenocarcinoma developing in a peripheral bronchial adenoma, W. Umiker & C. F. Storey, J. Thoracic Surg., October, 1952, 24: 420.*—(*R. E. MacQuigg*)

Esophageal Carcinoma.—Surgery for esophageal carcinoma produces disappointing survival and cure rates. With incurable tumors, swallowing may be restored by irradiation or by a shunting procedure, preferably antethoracic esophagoplasty. With cancer of the lower one-fourth of the esophagus, resection and esophagogastrostomy is the treatment of choice for resectable lesions and an intrathoracic shunt with a Roux-Y jejunal loop is preferred for inoperable lesions. For resectable tumors in the mid-esophagus, there are three operations, each with its own advantage: (*1*) resection and esophagogastrostomy, (*2*) resection and antethoracic esophagoplasty, and (*3*) resection and plastic tube reconstruction. For nonresectable tumors and in debilitated patients, antethoracic esophagoplasty may be offered with supplemental irradiation. Lesions near the apex of the thorax are best treated by antethoracic esophagoplasty with or without resection or irradiation. Good results are obtained by implanting a plastic tube in the mid-esophagus as advocated by Berman. In discussing this paper, Brewer pointed out that the early symptoms of esophageal malignancy are mild discomfort, soreness, or tightness in the thorax or upper abdomen. If the esophagus is considered, along with the lungs and heart, as a possible cause of these mild symptoms, early cancer may be detected. At first the dysphagia is not progressive and at times, for various reasons, the patient swallows better. In some cases, the proper diagnosis could have been made six months to one year earlier by thorough esophageal study when the symptoms were primarily epigastric in nature. The first patient whose esophageal carcinoma was resected with esophagogastrostomy in this country in 1938 by Adams and Phemister is still alive.—*Carcinoma of the esophagus, M. M. Ravitch, H. T. Bahnson, & T.N. P. Johns, J. Thoracic Surg., September, 1952, 24: 256.*—(*R. E. MacQuigg*)

Esophageal Rupture.—Esophageal rupture can often be easily diagnosed from the history and physical examination, by finding the diagnostic triad of rapid respiration, abdominal rigidity, and subcutaneous emphysema. If the diagnosis is in doubt, further proceedings may be used. Fluid aspirated from the chest cavity should be examined for food particles and hydrochloric acid; if either is present, the diagnosis is substantiated. A swallow of barium or Lipiodol followed by fluoroscopy will often outline the perforation or show a periesophageal pocket directly communicating with the pleural cavity. If doubt remains, dye can be given by mouth and recovered in the intrathoracic fluid. Esophageal rupture is a surgical emergency, and the diagnosis must be established immediately. Only those procedures absolutely necessary for that purpose should be done and, as soon as the diagnosis is established, a thoracotomy and repair of the rupture should be performed. Nothing is gained by procrastination, and the presence of shock should not delay surgery. After the chest is opened, the pleural cavity should be aspirated and the lung re-expanded. A feeding tube passed through the mouth or nose into the stomach may also be used for decompression. The shock in these cases is due to pain, hypoxia, and blood loss. The hypoxia is due to tension pneumothorax, pleural fluid, and poor respiration as a result of pain. During anesthesia, the patient has a high oxygen intake and, without pain, breathes better. Thoracotomy relieves the tension pneumothorax; the pleural fluid is removed, and the lung expanded. Prompt and complete expansion of the lung is important in preventing empyema. The repair may break down on the sixth to the tenth postoperative day. If this occurs, the open perforation must be drained. The fistula thus formed will often close in several weeks. The 4 patients so treated survived, in contrast to a mortality of almost 100 per cent in the older literature. In discussing this paper, Fryfogle reported a newborn girl who, shortly after a normal delivery, became dyspneic and cyanotic. Roentgenograms confirmed the diagnosis of tension pneumothorax, which was relieved by needle aspiration. Twelve hours later another crisis was relieved by aspiration of mucus from the chest. At thoracotomy, there was found total mucoid empyema and a rupture at the base of the esophagus perforating through the right pleura. A web directly across the base of the esophagus prevented air from entering the stomach. The rent in the esophagus was enlarged, the small web was cut out, and a tube was passed through the nose into the stomach. The perforation was then closed and the lung decorticated. The patient did well.—*Rupture of the esophagus, R. L. Anderson, J. Thoracic Surg., October, 1952, 24: 369.*—(*R. E. MacQuigg*)

Pulmonary Resection for Chronic Abscess.—From September, 1948, to March, 1952, 11 lobectomies and 7 pneumonectomies were performed in 18 patients with chronic pulmonary abscess. All gave a history of nontuberculous chronic discharge from the lung. The disease had lasted for three months to eight years; the patients were eight to forty-four years old. There were 12 men and 6 women. In 15, there was a history of pneumonia in the course of which the abscess had been detected. In 2 cases, foreign bodies were present. All the patients had been treated with antibiotics. Among the 11 lobectomy cases, one death occurred because of an anesthetic mishap. Three operative deaths occurred among the 7 patients subjected to pneumonectomy. The poor condition of patients accounted for this high death rate.—*Resecção pulmonar por abscesso crónico, J. S. Cavalcanti, Rev. brasil. med., September, 1952, 9: 618.*—(*A. A. Moll*)

Early SK-SD with Resections.—Following partial pulmonary resections, 6 patients were treated with streptokinase-streptodornase (SK-SD) in the early postoperative period. The enzymes were instilled through drainage tubes already *in situ* on the second to the eighth postoperative days. In no instance was a chronic fistula created nor was

there evidence of any deleterious effect on the underlying disease. All of the wounds have healed by first intention. Tubes must be in place when this material is used under these circumstances. Suction should be available and it may be necessary to introduce additional drain tubes.—*The use of SK-SD (Varidase) in the management of early postoperative partial pulmonary resections, C. T. Read, J. Thoracic Surg., September, 1952, 24: 284.*—(*R. E. MacQuigg*)

Traumatic Saccular Aneurysm.—The many crushing blows contributing to the ever increasing accident rate make the early recognition of traumatic aneurysm more and more important. Few cases of this complication have been reported; none of the patients survived longer than eleven months, and all were diagnosed at autopsy. Four new cases have been diagnosed during life and the patients have all survived a few months to seven years following their injury. Of the 4 new cases, 3 presented almost identical roentgenographic findings, which may aid in diagnosing this lesion in the future. There was a narrow band of increased density just to the left of the heart shadow. This density was attributed to blood dissecting along the aortic wall. Angiograms have been of great value in making a correct diagnosis when the condition of the patient permits. When the lesion has been diagnosed at an exploratory operation, it should be wrapped with Cellophane. This adds little to the difficulty of the procedure and may prevent future trouble.—*Traumatic saccular aneurysm of the thoracic aorta, R. K. Hollingsworth, W. W. Johnston, & J. F. McCooey, J. Thoracic Surg., October, 1952, 24: 335.*—(*R. E. MacQuigg*)

Roentgenograms and Pulmonary Dynamics.—In order to secure more complete data on thoraco-pulmonary dynamics, static roentgenographic methods may be combined advantageously with newer kymographic and diagraphic techniques used in collapse therapy. The changes made by Abreu and Lobo de Carvalho in Sabath's apparatus and by Seabra in Pollitzer's, as well as a new circular kymograph, still in the experimental stage, are of value in such studies.—*Métodos radiológicos de investigação da dinâmica toracopulmonar, D. Soares & J. Pinto Soares, Rev. brasil. med., June, 1952, 9: 386.*—(*A. A. Moll*)

ANTIMICROBIAL THERAPY

Isoniazid for Renal Tuberculosis.—Isoniazid improves the bladder lesions of renal tuberculosis. It causes the urine culture to become normal in some patients. It is not nephrotoxic. In patients with nitrogen retention, blood concentrations of the drug should be determined in order to prevent serious convulsions. Streptomycin and *p*-aminosalicylic acid have been used in 458 cases of genitourinary tuberculosis. Symptomatic improvement has been impressive during the five-year observation period. Medium-sized lesions, where only one calyx was involved, showed 40 per cent conversion of urine cultures after five years. Small lesions showed 80 per cent conversion. Inoperable, bilateral renal tuberculosis was treated with one to three years of intermittent combined therapy. Unilateral, destructive renal tuberculosis was treated by nephrectomy.—*Caution necessary in the treatment of renal tuberculosis with isoniazid, J. K. Lattimer, J.A.M.A., November 8, 1952, 150: 981.*—(*H. Abeles*)

Isoniazid and Iproniazid Therapy.—Iproniazid has been given to 101 patients with pulmonary tuberculosis for three to seven months, isoniazid to 65 patients for two to five months, and streptomycin and a hydrazine derivative to 9 patients for three months. The dosage of iproniazid was 4 mg. per kg. of body weight per day. Isoniazid was employed in doses of 4 and 8 mg. per kg. Therapy with the hydrazine drugs has been characterized by rapid and marked amelioration of systemic manifestations of tuberculosis. Cough and expectoration were significantly reduced. Beneficial roentgenographic changes were seen in approximately 60 per cent of the patients. Approximately 12 per cent of the

patients deteriorated. The sputum became negative for tubercle bacilli in 25 per cent of the patients. Of the 175 patients, 7 died; 2 of them had massive hemoptysis. Significant toxic side effects, especially of the nervous system, occurred infrequently at the recommended dosage levels, but were more frequent and potentially hazardous at higher levels. Side-effects were more common in elderly persons, in patients with unstable personalities, convulsive tendencies, or a history of psychosis, and in the presence of anemia.—*Treatment of pulmonary tuberculosis with hydrazide derivatives of isonicotinic acid, I. J. Selikoff, E. H. Robitzek, & G. G. Ornstein, J.A.M.A., November 8, 1952, 150: 973.—(H. Abeles)*

Isoniazid for Pulmonary Tuberculosis.—After other treatment had been tried at a tuberculosis sanatorium, 39 patients with moderately or far advanced pulmonary tuberculosis were given routinely doses of 3 mg. of isoniazid per kg. of body weight, and occasionally doses of 5 mg. per kg. After a follow-up period of 120 days, the general condition, nutrition, and stethoscopic findings improved in many cases. This improvement became evident during the first thirty days, increased after sixty days, and was maintained to a large extent to the end of the observation period. The sputum progressively but not constantly tended to become negative. Roentgenographic shadows tended to clear, reflecting changes in the exudative character, appearance, and size of cavities with a bronchial mechanism. The patients remain under observation.—*Resultados clínicos del empleo de los derivados del ácido iso-nicotínico, O. García Rosell, C. Arellano, J. Arredondo, H. Arriola, P. Baldeón, S. Bonilla, M. Costa, S. Hauco Vizcardo, C. López, V. Narváez, S. Neyra, E. Olivares, E. Osma, V. Reyes, V. Tejada, & A. Vargas, Rev. tuberc., Habana, January-June, 1952, 12: 56.—(A. A. Moll)*

Laboratory Studies of Isoniazid.—Isoniazid exhibited bacteriostatic action on the H37Rv strain of tubercle bacilli at concentrations of 0.005 γ per cc. *in vitro* and inhibited growth completely at concentrations of more than 0.5 γ per cc. Its bacteriostatic power *in vitro* was more than fifty times greater than that of dihydrostreptomycin. Continued exposure of fifty days to 0.08 γ per cc. killed the H37Rv strain *in vitro* in a fluid medium. Of 15 tuberculous patients treated with isoniazid, 8 (53 per cent) showed fluctuations in streptomycin sensitivity. This change consisted in a reversion of resistant strains to sensitivity or decreased resistance in 33 per cent and change from resistance to sensitiveness and then the reverse in 20 per cent. Following a treatment period averaging 126 days, 4 of 7 cases yielded isoniazid-resistant bacilli. Growth in the presence of more than 1 γ per cc. was taken as evidence of resistance.—*Pruebas de susceptibilidad a los antibióticos durante los ensayos de laboratorio con isoniacida, J. Gálvez B., Rev. tuberc., Habana, January-June, 1952, 12: 12.—(A. A. Moll)*

Isoniazid in Guinea Pig Tuberculosis.—Two groups of 15 guinea pigs each, previously infected with tuberculosis, were treated parenterally with isoniazid in an aqueous solution. The first group was treated during the first thirty days after infection and the second from the thirtieth to the sixtieth days. All animals were killed at the end of the experiment, together with 2 groups of 5 nontreated infected guinea pigs as controls. The first treated group showed no nodules at the inoculation site and no systemic lesions. In the animals treated belatedly, caseous lesions about the inoculation nodule and satellite nodes failed to exhibit significant changes; the spread to other organs was discrete and less extensive than in the control animals. Skin ulcers developing in the inoculation site in the group treated belatedly healed as a result of the treatment. Inoculation with apparently normal spleens from guinea pigs in the second group revealed the presence of virulent live organisms in 70 per cent. In all animals treated with isoniazid, sensitivity to tuberculin decreased significantly. No toxic effects referable to the drug were detected.—

Acción de la isoniacida en la tuberculosis experimental del cobayo, R. Vargas Machuca & J. Campos Rey de Castro, Rev. tuberc., Habana, January–June, 1952, 12: 31.—(*A. A. Moll*)

Tuberculous Meningitis.—Of 270 patients with tuberculous meningitis seen between June, 1950 and May, 1951, 70 were admitted to the hospital and studied. Of these patients, 61 were fifteen years of age or younger and most were between one and three years of age. The commonest early symptoms were fever, an irritable temper, loss of appetitie, headache, and vomiting. Only about one-fourth of the patients received therapy during the first week of illness and more than half were delayed for two weeks or longer before treatment with streptomycin was started. Diagnostic lumbar punctures were performed on all patients, but cultural or animal studies were not done on any of the specimens. In the past, more than 90 per cent of cerebrospinal fluid specimens with a cell count of 50 to 100 per cu. mm., a sugar level below 45 mg. per 100 cc., and a marked web formation have usually proved positive by guinea pig inoculation. Streptomycin was administered intramuscularly and intraspinally as follows: (*1*) children up to five years of age received 20 to 100 mg. intrathecally five to ten times and 0.3 to 0.5 gm. intramuscularly for three to four months; (*2*) older children received 40 to 150 mg. intrathecally seven to fifteen times and 0.6 to 1.0 gm. per day intramuscularly for three to four months; (*3*) adults received 50 to 200 mg. per injection intrathecally ten to twenty times and 1.2 to 2.0 gm. per day intramuscularly for three to four months. Intrathecal injections were given once or twice each week. The mortality rate for the entire series was 36 per cent, the greatest percentage of fatalities being in adults more than 25 years old, in infants less than two years, and in those who had received previous streptomycin therapy for non-meningeal tuberculosis. The period of observation for this series of recovered cases varied from six to eighteen months without any recurrence of tuberculous meningitis.—*Therapy of tuberculous meningitis with special reference to streptomycin: A study of 70 cases with 36 per cent mortality, F. D. Zau, Y. W. Yui, T. H. Hu, L. Wong, & Y. K. Zau, Chinese M. J., July–August, 1952, 70: 299.*—(*L. Hyde*)

Streptomycin for Pulmonary Tuberculosis.—Streptomycin or dihydrostreptomycin was used in 146 cases of pulmonary tuberculosis in a Bahía hospital. It was associated with *p*-aminosalicylic acid in 43 cases, which were as a whole more serious. Despite this, results were 10 per cent better in the latter group. Of the entire series, 59 patients became bacteriologically negative and 97 improved generally. Results were significantly better among men than women. In 12 of 105 cavitary cases, cavities closed, and in 36 decreased in size. As in other series, exudative cases, acute exacerbations, and certain cavitary types benefited most. Use of both antibiotic and chemotherapeutic agents should be strictly controlled by definite indications. Their employment should be avoided when surgical treatment is considered essential.—*Resultado do tratamento da tuberculose pulmonar pela estreptomicina em doentes internados na "Fundação Hospitalar Octavio Mangabeira", F. Serra & D. Alvares, Clín. tisiol., May–June, 1952, 7: 353.*—(*A. A. Moll*)

Streptomycin for Pulmonary Tuberculosis.—A review of 160 patients with pulmonary tuberculosis, treated with streptomycin between 1947 and 1950, showed that the results were best in cases of predominantly exudative lesions, definitely acute and more recent cases, and less advanced lesions. Patients without cavities responded better than those with cavities. Streptomycin yielded better results when used with other therapeutic measures than when used alone. The antibiotic widened surgical indications and improved the results of surgery. Younger patients tolerated the drug better than aged persons. Incomplete data about streptomycin resistance limited the usefulness of this agent.—*A estreptomicina na tuberculose pulmonar, M. Pires, Rev. brasil. med., August, 1952, 9: 551.*—(*A. A. Moll*)

Antibiotics for Surgical Tuberculosis.—Streptomycin was given preoperatively to 75 patients before phrenic avulsion; 18 patients benefited from the medication, 27 had mediocre results, and 27 did not benefit. Of the 22 patients given the drug postoperatively, many benefited. Of 17 patients who died, 6 had moderately advanced, and 11 far advanced, tuberculosis; 10 had received streptomycin. Streptomycin was given preoperatively to 61 patients subjected to thoracoplasty, with uncertain results. It was used postoperatively in 45 thoracoplasty cases because of reactivation of pre-existing lesions, local extension, or as a preventive; results were generally favorable. Of 65 cases of bronchial lesions treated with streptomycin, 28.7 per cent did not show any change, 21.3 per cent got worse, and 50 per cent improved. In 9 cases subjected to Monaldi's operation and in 10 pneumonoplasty cases, streptomycin seemed to help. In 28 pulmonary resection cases, streptomycin helped to make the procedure practicable. Among 120 pneumolysis cases, 18 received streptomycin either before or after the operation. The drug permitted the use of pneumothorax in more patients and decreased the incidence of empyema. In intrapleural pneumolysis cases, streptomycin did not seem to influence the development of complications which were usually related to the surgical technique. While the drug should not be used routinely as a preoperative preventive measure, it should be given for postoperative exacerbations. Before major operations like thoracoplasty and pulmonary resection, the streptomycin sensitivity of the bacilli should be determined. If drug resistance is found, *p*-aminosalicylic acid (PAS) or a combination of PAS and streptomycin should be tried.—*Antibióticos en el tratamiento de la tuberculosis: Cirugía en la tuberculosis pulmonar y antibióticos, M. Martínez, E. Rodríguez, E. Romero, & A. Llona, Clín. tisiol., May-June, 1952, 7: 271.*—(*A. A. Moll*)

Treatment of Tuberculous Pericarditis.—A series of 34 cases of tuberculous pericarditis admitted to the Philadelphia General Hospital from 1941 to 1952 was divided into a group of 20 patients who did not receive antimicrobial therapy and a group of 14 patients who did. These 2 groups were comparable in all other respects. Streptomycin and possibly *p*-aminosalicylic acid and isoniazid reduce the mortality rate. Thus, 19 (90 per cent) of the 20 untreated patients died, whereas only 8 (43 per cent) of the 14 treated patients succumbed. Two of the 6 surviving patients are still under treatment; 4 are discharged and well after five to thirty-six months. Although the mortality rate was lowered by antimicrobial therapy, half of the patients who died did so within three months whether receiving antimicrobial agents or not. There was a significant difference between the untreated and treated patients with respect to reduction of cardiac size on roentgenogram where serial films were available; 25 per cent of 12 untreated cases and 75 per cent of 12 treated cases were reduced. There was relatively little difference with respect to the return of the electrocardiogram to normal where serial tracings were taken; 11 per cent of 9 untreated cases and 30 per cent of 10 treated cases returned to normal. When electrocardiographic improvement occurred, it was slow. No conclusions were warranted concerning the optimal regimen of therapy or the long-term results.—*Tuberculous pericarditis with effusion: The impact of antimicrobial therapy, J. B. Shapiro & W. Weiss, Am. J. M. Sc., March, 1953, 225: 229.*—(*W. J. Steininger*)

Decrease of Organisms in Streptomycin-Treated Cavities.—Among 66 patients with pulmonary tuberculosis and cavities, the use of streptomycin brought about a decrease in bronchial discharge and occasionally, in 16 cases, conversion of the sputum to negative, even when tubercle bacilli persisted in the gastric contents. In 5 of 9 patients with open cavities, cultures became negative and in 2 other cases the cavities closed under treatment with streptomycin.—*Valorização da pesquisa de bacilos nas secreções de tuberculosos cavitários submetidos ao tratamento pela estrepto-*

micina, M. Tavares de Lima Filho, B. de Costa Lima, M. de Mello Faro, H. Prudente Junqueira Reis, & D. Abuchala, Clín. tisiol., May-June, 1952, 7: 318.—(A. A. Moll)

Placental Toxicity of Streptomycin.—Experiments with pregnant guinea pigs showed that streptomycin and dihydrostreptomycin had a toxic effect on the placenta characterized by the development of hemorrhagic necrosis. Single daily doses of more than 100 mg. per kg. of body weight caused abortion in all of the animals within one week. Daily doses of 25 to 50 mg. per kg. of body weight resulted in abortion in some animals. Animals given doses of 10 mg. per kg. did not abort. All the surviving young were normal. In the majority of the fetuses the serum concentrations of streptomycin and dihydrostreptomycin were between 2 and 4 per cent of that in the mothers. The toxic effect of streptomycin and dihydrostreptomycin on the placenta is more marked and histologically more easily demonstrated than that on the eighth cranial nerve of the guinea pig.—*The toxic effects of streptomycin and dihydrostreptomycin in pregnancy, illustrated experimentally, N. Riskaer, E. Christensen, & H. Hertz, Acta tuberc. Scandinav., 1952, 27: 211.—(K. T. Bird)*

LABORATORY STUDIES

Middlebrook-Dubos Hemagglutination Test.—To evaluate the reproducibility, sensitivity, and specificity of the hemagglutination test, 372 sera obtained from 199 persons were examined. The effect of intracutaneous PPD upon the serologic titers was also measured. Sera were tested in groups of 10, using Lederle's OT concentrated to four times the international standard. By defining a significantly elevated titer as 1:64, the test was sensitive enough to indicate clinical infection in 70 per cent of 30 tuberculous patients. A titer of 1:32 (doubtful significance) or higher was found in 87 per cent of the tuberculous patients and 4 per cent of the nontuberculous persons. Low titers were found in tuberculous patients who were dying of their disease and in healthy persons. The test was acceptably specific for tuberculosis if a titer of 1:64 was considered critical. Intracutaneous PPD caused significant titer changes in patients with positive or negative cutaneous reactions. Titer rises of 1:64 were observed only in patients with positive cutaneous reactions.—*An evaluation of the Middlebrook-Dubos hemagglutination test, E. F. Rabe & W. S. Spicer, Jr., J. Lab. & Clin. Med., January, 1953, 41: 98.—(F. G. Petrik)*

Pulmonary and Bronchial Arteries.—The pulmonary arteries were injected with radiopaque suspensions in 27 lungs or lobes and the bronchial arteries in both lungs from 7 subjects, and the specimens were subsequently radiographed. When the pulmonary arteries were injected, filling of the arteries did not obscure the main vessels and both arterioles and larger vessels could be studied. In normal lungs no anastomosis between pulmonary and bronchial arteries could be demonstrated radiologically. Small solid lesions up to 3.5 cm. in diameter did not affect the pattern of the arterial tree. In areas of pulmonary fibrosis the arteries had few branches, were narrowed and crowded together. The main arteries persisted, the smaller being lost. Other changes occurred in collapse and emphysema. The bronchial arteries were tortuous and anastomosed with each other. Demonstrable precapillary anastomoses with pulmonary arteries indicated enlargement of the bronchial arteries, and this occurred in association with long-standing infection, particularly bronchiectasis. The contrasting pattern of pulmonary, bronchial, and pleural vessels and the anastomoses between them may become important if angiography is developed in the study of pulmonary disease (Author's summary).—*Injected pulmonary and bronchial arteries, C. P. Silver, Brit. J. Radiol., December, 1952, 25: 623.—(L. Hyde)*

BCG Assay.—Direct agar microscopy was used to study the germination and growth characteristics of BCG cultures on Dubos oleic-acid–albumin–agar medium. Four-day-old vaccines stored under various conditions

and one lyophilized specimen were examined. Bacterial counts were made and, when single organisms could be seen, they were divided into 2 categories according to morphology. When colonies formed, their size and density was estimated. The proportion of non-germination was estimated. Growth was also estimated morphologically by observing what proportion of the organisms changed structurally towards disintegration or division. Although the two methods gave comparable results, the counting technique was more quantitative and needed less experienced interpretation. Both methods are serviceable for assaying BCG vaccines. The characteristics varied in the eight different vaccines tested. Storage at room temperature impaired the capacity to grow; storage at 3° to 5°C. did not alter the vaccine. The lyophilized specimen did not grow.—*Germination and growth of BCG vaccine followed by the direct agar microscopy on Dubos media: II, H. C. Engbaek & J. Orskov, Acta path. et microbiol. Scandinav., 1952, 31: 360.*—(*H. G. Turner*)

Laboratory Time Saver.—Adding a 10 per cent solution of ferric sulfate to voluminous fluid specimens, after they have been buffered and acidified, shortens the time required to prepare such specimens for culture or guinea pig inoculation. Subsequent neutralization produces a precipitate which carries tubercle bacilli with it. After standing two hours, the supernatant fluid is discarded, reducing both the volume to be centrifuged and the time required. Total centrifuging time until the specimen is ready for inoculation is no longer than fifteen minutes. Trial specimens gave comparable results with the old and the new methods. The ferric sulfate method was then adopted and used for three years, during which it yielded approximately the same percentage of positive results as did the old method in previous years. The method is at least as accurate as others and is justified by its time-saving value.—*Demonstration of tubercle bacilli in gastric lavage and urine, O. Sievers, Acta path. et microbiol. Scandinav., 1952, Supplement 93: 422.*—(*H. G. Turner*)

Effect of Light on BCG.—After 2 preliminary studies had shown the destructive effect of sunlight and daylight on BCG vaccine, it was prepared by a technique which avoided exposure of the vaccine to daylight. Since adoption of this technique, there has been no significant variation in the content of viable bacilli among the batches of vaccine, in contrast to previous experience. Samples of each batch were exposed to daylight through a double glass window and the content of viable organisms was found to decrease according to the duration and intensity of light exposure. This destruction by light may account for the large variations in vaccines in the past.—*Preliminary report of laboratory investigations on the effect of light on BCG vaccine, K. Tolderlund, Acta path. et microbiol. Scandinav., 1952, Supplement 93: 299.*—(*H. G. Turner*)

Standardizing BCG with Tetrazole.—Reduction of 2,3,5-triphenyl-tetrazole chloride by living BCG strains is a simpler method for standardizing this vaccine than Thunberg's methylene blue reduction technique. The number of living organisms is determined quantitatively by assaying colorimetrically the amount of red-tinted formazan from the reduced tetrazole. There is a linear ratio between the amount of formazan and living BCG; 1.0 mg. of live BCG reduces 6.4 γ of tetrazole. A BCG unit is defined as the amount of BCG which reduces 320 γ of 2,3,5-triphenyl-tetrazole chloride. Tetrazole is not reduced by BCG which have been killed by heat, direct sunlight, or 2 per cent formol. The survival time of BCG in the refrigerator varies according to the method used in making the vaccine; it is shorter when the organisms are more scattered.—*Padronização do B.C.G. II., D. M. Cardoso, G. Nazário, Jandira P. do Amaral, & W. F. Almeida, Rev. brasil. tuberc., September-October, 1952, 20: 583.*—(*A. A. Moll*)

Tests in Hamsters of BCG Potency.—Recent experiments indicate that the controls of BCG potency should include subcutaneous and intraperitoneal inoculation in guinea pigs

and in hamsters. The dosage should be 30 mg. subcutaneously and 25 mg. intraperitoneally. Electron microscope studies confirmed the findings by Reed, Rosenthal, and Reed regarding appearance of BCG preparations submitted to vaporization.—*Notas prévias: B.C.G., M. J. Pereira Filho, Rev. brasil. tubere., November–December, 1952, 20: 713.*—(*A. A. Moll*)

Cortisone and Tuberculosis.—In order to determine whether cortisone aggravates tuberculous lesions and increases the number of bacilli and whether it affects the activity of phagocytes, the hormone was given to guinea pigs with acute tuberculosis and to mice with chronic disease. Besides the usual histologic observations, cultures were made by crushing the left lung or spleen of the animal, suspending the tissue in saline solution, and planting serial dilutions of an aliquot on slants. Bacterial counts after four weeks' incubation showed that cortisone increased the number of bacilli after the twelfth day in acute tuberculosis. This was confirmed histologically; in addition, there was a less intense lymphocytic reaction than in the control animals. In the animals with chronic lesions, the intense lymphocytic reaction disappeared after fourteen days of cortisone administration, and the cells in the lesions developed indistinct borders. Colony counts showed a marked increase in the number of bacilli; histologically, the bacilli were in colony-like groups, chiefly within phagocytes but occasionally within swollen cells of the alveolae. Marked necrosis was not seen. The spleens were small in the treated animals of both series, with atrophy of the lymphoid apparatus and, in chronic disease, some pre-amyloid deposits as well. In the acutely ill animals, spleen cultures were negative through the tenth day in both treated and untreated groups and were positive thereafter; dissemination was unrelated to treatment. Spleen bacterial counts in the chronically ill group did not show a definite pattern.—*Effect of cortisone on the tuberculous infection in mice and guinea pigs, H. C. Engbaek, T. Friis, & G. Teilum, Acta path. et microbiol. Scandinav., 1952, 31: 317.*—(*H. G. Turner*)

Cortisone and Tuberculin Cytotoxicity.—Cortisone suppresses the local reaction of hypersensitive animals and man to the intracutaneous injection of tuberculin. In this type of hypersensitivity, mononuclear cells play an important part. The effect of cortisone on the reaction of hypersensitive cells to PPD can be studied in tissue cultures without interference from blood-borne substances, vascular effects, or nonspecific tissue repsonses. PPD prevents the migration of hypersensitive macrophages which tend to become rounded, small, and granular. Tissue culture of spleens from tuberculous animals were grown in roller tubes, using plain nutrient medium or nutrient containing cortisone acetate for the first twenty-four hours. These media then were replaced respectively by nutrient containing PPD or nutrient containing both PPD and cortisone. Splenic tissue from normal guinea pigs was treated in a similar manner. Other compounds tested in the same manner as cortisone included desoxycorticosterone glucoside, hydrocortisone, the pure alcohol of cortisone, and an estrone. Cortisone, 100 γ per ml., and hydrocortisone inhibited the cytotoxic effect of PPD upon the hypersensitive macrophages. Pretreatment of the cells for twenty-four hours with the hormones before exposure to PPD was essential to obtain this result. Neither cortisone nor PPD inhibited the migration of normal macrophages in the amounts used. Less than 80 γ of PPD per ml. failed to cause toxic changes in sensitive cells; concentrations greater than 100 γ per ml. were slightly toxic to normal cells. It would seem that hypersensitive macrophages were altered by cortisone so that they were no longer specifically reactive to PPD. The specificity of the cortisone action is emphasized by the failure of two other steroids to have protection against PPD.—*The inhibition by cortisone of the cytotoxic activity of PPD on tuberculin hypersensitive cells in tissue culture, R. H. Leahy & H. R. Morgan, J. Exper. Med., December 1, 1952, 96: 549.*—(*J. S. Woolley*)

Corticotropin and Cortisone in Amyloid Degeneration.—The concentration of blood glob-

ulins and antibodies changes in amyloid degeneration and these blood proteins are influenced by corticotropin (ACTH) or cortisone. To study possible exciting or restraining effect of these hormones on the development of amyloidosis, amyloid degeneration was produced experimentally in white mice by subcutaneous injections of sodium caseinate. Of the 4 groups of animals given caseinate, 2 were controls; one group received corticotropin; and one, cortisone. In the corticotropin group, all the animals developed amyloid as against 40 per cent of the controls; and in the cortisone group, 88 per cent in contrast to 21 per cent of the controls. By a special histoquantitative technique the amount of amyloid in the spleen was found to be much increased in the hormone-treated animals as compared to the controls and there was a different histologic picture. Albumin decreased, alpha and beta globulins increased in all groups. The hormones may retard antibody formation which promotes the antigen-antibody reaction and thus increases the precipitation of amyloid. A decrease in lymphocytes and an increase in neutrophils was also noted at the time of production of the amyloid.—*The effect of ACTH and cortisone on experimental amyloid degeneration, J. Latvalahti, Acta path. et microbiol. Scandinav., 1952, Supplement 93: 81.—(H. G. Turner)*

Lung Damage by Oxygen.—To determine why hypophysectomy decreases the adverse response to oxygen at high pressure, 50 young male albino rats were exposed to oxygen at approximately 80 pounds of pressure for several minutes once daily over several days or weeks. The results confirmed the earlier finding that non-hypophysectomized animals had a more rapid onset of the reaction to high-pressure oxygen and much more severe adverse neuromuscular, central nervous system, and pulmonary effects than had hypophysectomized animals. The lungs of the unoperated animals were liver-like over much and (in many cases) over almost all of both lungs which sank in a fixing solution. Passive congestion was prominent; hemorrhage, especially by diapedesis into the alveoli, atelectasis, edema, bronchiectasis, inflammatory reactions, and emphysema were common. In contrast, the lungs of hypophysectomized animals were less severely affected, some remaining entirely normal even though exposed more often and longer than the unoperated animals. The pulmonary damage induced by oxygen at high pressure contributed greatly to the various adverse changes and was most commonly the immediate cause of death. The greater resistance of the hypophysectomized animals to high pressure oxygen would appear, therefore, to be due largely to the pulmonary protection which hypophysectomy affords. Results indicate that the increased resistance of hypophysectomized animals is due to a decreased production of adrenal cortical factors. Hypophysectomy failed to provide a similar protection against the pulmonary injury inflicted by intratracheally inoculated pneumococcus.—*Influence of hypophysis on pulmonary injury induced by exposure to oxygen at high pressure and by pneumococcus, J. W. Bean & P. C. Johnson, Am. J. Physiol., November, 1952, 171: 451.—(G. C. Leiner)*

Intestine and Respiration.—In dogs anesthetized with sodium pentothal, occlusion of the artery and vein of intestinal loops, completely isolated except for the extrinsic nerves, transiently augmented breathing. Application of sodium cyanide to the peritoneal surface of the loop while the vessels were occluded caused a small initial depression of breathing followed by a pronounced augmentation. Reversal of these effects was hastened by rinsing the tissue in Tyrode's solution. Releasing the vessels increased breathing again which was attributed to the release into the general circulation of cyanide, accumulated metabolites, and possibly unknown substances from the intestinal loop. The receptors involved are activated by chemical changes which occur in the tissues as a result of disturbances in the blood flow, by chemical alterations in the blood itself, and by chemical changes in the intraperitoneal space acting directly on peritoneal surfaces. These respira-

tory reactions, which were demonstrated to be of reflex origin, serve to supplement other mechanisms in respiratory regulation (Author's summary).—*Intestine in reflex chemical control of breathing, J. W. Bean, Am. J. Physiol., December, 1952, 171: 522.*—(*G. C. Leiner*)

Atelectasis and Pulmonary Blood Flow.—Atelectasis was produced in dogs by bronchial ligation. The blood flow in the atelectatic lung was reduced until it was zero to 34 per cent of the total blood flow as the pulmonary vascular resistance increased. Maximal effects were observed 10 to 45 minutes after the ligation. The blood flow was reduced by atelectasis far more than by unilateral nitrogen breathing, suggesting that the mechanical factor of collapse is important in reducing the flow in atelectasis. The changes must be in the small vessels of the lung. In the discussion, Finnerty stated that in Crafoord's clinic the oximeter showed a marked fall in the oxygen saturation of peripheral blood in patients when the operated lung was allowed to collapse. Occlusion of the pulmonary artery to the deflated lung obliterated this "shunt" and the peripheral saturation rose immediately to normal or near normal levels. Clinically, prolonged collapse of an operated lung which has an intact pulmonary blood flow probably leads to carbon dioxide retention and respiratory acidosis as well as hypoxemia. These changes can be reversed by occluding the pulmonary artery. Thus, closing the "shunt" sometimes causes such marked improvement that an operative procedure can be completed after it was to be abandoned. Peters suggested that all the nitrogen should be removed from the lungs and blood by oxygen breathing before starting endobronchial anesthesia so that the lung will immediately collapse following bronchial occlusion.—*The effects of atelectasis on pulmonary blood flow in the dog, R. M. Peters & A. Roos, J. Thoracic Surg., October, 1952, 24: 389.*—(*R. E. MacQuigg*)

Tuberculosis Induced by Droplets.—When animals are infected with many tubercle bacilli by subcutaneous or intravenous injections, each appears to respond according to its so-called native resistance and the nature of the infecting organisms. But when such animals inhale aerosols containing few bacilli, the early results are at variance with this concept. To illustrate this, separate groups of rats, mice, hamsters, and guinea pigs were exposed to aerosols containing virulent tubercle bacilli of human or bovine strains. The stages of tubercle development during the first three weeks were studied in animals which had inhaled relatively large numbers of bacilli. Mice were estimated to have inhaled 500, rats and hamsters 2,000, and guinea pigs 5,000 viable organisms. Tubercle development after twenty-one days was studied in animals which had inhaled less than 50 organisms. The initial reaction of all animals was uniform for approximately three weeks. During the fourth week and thereafter, the rate and pattern of tubercle formation were distinctive for each species of host and strain of parasite. Initially, the disease progressed through three distinct stages: (*1*) growth of the bacilli in alveolar macrophages which collected about them in the lungs; (*2*) acute inflammatory reactions about these foci, with necrosis of the alveolar macrophages and sharp reduction in the number of bacilli; (*3*) transformation of the inflammatory reaction into a less active process which slowly became characteristic of each host-parasite combination. In rats and mice, gross disease was confined to the lungs. Hamsters proved resistant to human but susceptible to bovine tubercle bacilli. Guinea pigs were susceptible to both strains but particularly to the bovine type. The uniform initial response indicates that these species do not differ in their inherent resistance to inhaled human or bovine tubercle bacilli; they differ widely in their capacity to acquire resistance to these organisms, as shown by the variation in the later stages of the disease. —*Tuberculosis induced by droplet nuclei infection: Initial homogeneous response of small mammals (rats, mice, guinea pigs, and hamsters) to human and to bovine bacilli and the rate and pattern of tubercle development, H. L. Rat-*

cliffe & V. S. Palladino, J. Exper. Med., January 1, 1953, 97: 61.—(J. S. Wooley)

Use of Carbohydrates by Tubercle Bacilli.—Because previous studies have not defined the carbohydrate requirements of *M. tuberculosis* var. *hominis*, growth studies were conducted, employing a large variety of carbohydrates and alcohols in a wide range of concentration with a strain of *M. tuberculosis* of high standard virulence and a technique which would permit estimation of the growth rate. The effect of 24 carbohydrates and polyhydric alcohols on the growth of the H37Rv strain was tested, utilizing the small inocula method in modified Proskauer and Beck medium, from which the glycerol was omitted. The bacilli, regardless of the concentration of substrate or the size of the bacterial inoculum, could not use for growth any of the 24 carbohydrates or polyhydric alcohols except glycerol, glucose, and possibly maltose. Growth in the medium containing glycerol was more rapid than in the same medium containing glucose or maltose, and more rapid in the medium containing glucose than in that containing maltose. Glycerol also supported growth in a wider range of concentrations than either glucose or maltose.—*Studies on the metabolism of Mycobacterium tuberculosis: I. The effect of carbohydrates and alcohols on the growth of Mycobacterium tuberculosis variety hominis, G. P. Youmans & A. S. Youmans, J. Bact., January, 1953, 65: 92.—(F. G. Petrik)*

Intermediate Carbohydrate Metabolism of Tubercle Bacilli.—To investigate the intermediate metabolism of carbohydrates by virulent tubercle bacilli, the rate of growth of the H37Rv strain was measured in a synthetic medium containing compounds related to the Kreb's tricarboxylic acid cycle as a sole source of carbon. The evidence obtained lends support to the theory that a tricarboxylic acid or similar cycle is involved in the carbohydrate metabolism of *M. tuberculosis* var. *hominis* since pyruvic, acetic, oxalacetic, oxalosuccinic, and α-ketoglutaric acids supported the growth of the H37Rv strain. These results are striking when one considers (*1*) the very few carbon sources which support the growth of this strain, (*2*) that the rates of growth obtained with these compounds were similar to the rate obtained in the presence of the best carbon source, glycerol, and (*3*) that these compounds supported the growth of the smallest inoculum (10^{-6} mg.) employed. Although other acids of the Kreb's cycle, such as citric, cis-aconitic, isocitric, succinic, fumaric, and malic acids, did not allow growth, the results do not exclude the operation of such a cycle since the penetration of these substances into the bacterial cell must be considered. It has been reported that clarified extracts of the H37 strain consume oxygen in the presence of citrate or isocitrate when methylene blue was employed as a hydrogen carrier. Fumarase and malic dehydrogenase have been found in soluble preparations from the H37 strain, but succinic dehydrogenase has not. If these enzymes are present and function in the metabolism of the H37Rv strain, many of the negative results obtained in the present study may be due to the inability of the substrates to penetrate the bacterial cell.—*Studies on the metabolism of Mycobacterium tuberculosis: II. The effect of compounds related to the Kreb's tricarboxylic acid cycle on the growth of Mycobacterium tuberculosis var. hominis, A. S. Youmans & G. P. Youmans, J. Bact., January, 1953, 65: 96.—(F. G. Petrik)*

Glucose Dissimilation by Tubercle Bacilli.—To determine whether certain substrates which may occur in the dissimilation of glucose to pyruvic acid support the subsurface growth of the H37Rv strain of tubercle bacilli, the small inocula method was used to determine the rate of growth. Theoretically, unless cell permeability is a limiting factor, an intermediate metabolic product should stimulate to a similar degree, not only the rate of growth, but also the growth of the same small inocula as the parent substrate. The results show that gluconic acid and glucose stimulated the growth of the bacilli to an approximately

similar degree. In contrast, the various substrates which are involved in the glycolytic process did not support growth appreciably. Pyruvic acid and lactic acid, end products in the oxidation of glucose, stimulated the rate of growth to a greater degree than did glucose, suggesting perhaps the presence of a more efficient terminal metabolic process for this strain.—*Studies on the metabolism of Mycobacterium tuberculosis: III. The growth of Mycobacterium tuberculosis var. hominis in the presence of various intermediates of the dissimilation of glucose to pyruvic acid, A. S. Youmans & G. P. Youmans, J. Bact., January, 1953, 65: 100.*—(*F. G. Petrik*)

PUBLIC HEALTH AND EPIDEMIOLOGY

Roentgenography of Chest.—It has been shown that there is considerable inter-observer and intra-observer error in the interpretation of chest films. Concern was felt that less attention was being paid to observer error in Great Britain than in the United States. To compare the results of observers reading the same sets of films in both countries, 89 films taken in Great Britain were selected. These contained a suitable proportion of films showing minimal disease. Six different readers viewed the films, each on two separate occasions. The films were read as either "active" tuberculosis, "clinically significant" tuberculosis, or negative. The films were sent to San Francisco, where 3 readers repeated the performance. The results were then reviewed in each country. British readers called a smaller proportion of the films positive for tuberculosis than did the American readers. However, more "clinically significant" cases were reported by the British readers. Inter-individual disagreements on "tuberculosis present" were lower for the British than for the American readers. Inter-individual disagreement on activity was generally low, but higher for the British. It was concluded that there was no considerable difference between the American and British readers in the accuracy of the interpretation of the films.—*Observer error in the interpretation of chest films, H. L. Cochrane & L. H. Garland, Lancet, September 13, 1952, 2: 505.*—(*A. G. Cohen*)

Tuberculosis Beds in Brazil.—An isolation hospital, with beds for tuberculous patients, was opened at Campos in 1791 but until approximately 1838 such patients were usually cared for with others in the hospitals of Brazil. Separate tuberculosis pavilions were first opened in different parts of the country after 1860. The first sanatoriums date from 1920 and 1924. Data as to the number of beds available at different dates are unsatisfactory; they lump beds planned, under construction, or not yet in operation with those actually in use. The number of beds in public and private institutions, in sanatoriums, hospitals, and other establishments has risen from an estimated 3,422 in 1935 to 11,654 in 1945 and 30,122 in 1950. Only approximately one-half of them are, however, now in active use. Among 43,428 patients admitted from 1947 to 1950 at tuberculosis hospitals, advanced cases averaged 86 per cent.—*Leitos para tuberculosos do Brasil, O. Revel Perrone, Rev. brasil. tuberc., November–December, 1952, 20: 791.*—(*A. A. Moll*)

Tuberculosis Beds in Brazil and BCG.—There were in Brazil in 1951 two state capitals, which are also the largest cities in the country, with tuberculosis death rates above 300 per 100,000 inhabitants; 8 capitals with rates above 200; 7, including the Federal District and S. Paulo, with rates above 100; and only 3 with rates under 100 per 100,000 inhabitants. The present number of beds for a population approaching 53 million is 34,730, approximately one-half of which are either only planned, under construction, or not in use yet. Only 5 states in the country, including S. Paulo, with 12,166 beds, and the Federal District, with 4,128 beds, have more than 1,000 beds. In the face of this inadequacy, more dependence should be placed on preventive measures, and especially BCG. Dosage of this vaccine in Brazil has been increased lately to a single dose of 0.1 gm.—*A premunição pelo B.C.G. na Campanha Nacional*

contra a Tuberculose nos Estados Unidos do Brasil, M. J. Pereira Filho, Rev. brasil. tubérc., November–December, 1952, 20: 653.—(*A. A. Moll*)

Tuberculosis Mortality in Rio de Janeiro.—Approximately a century ago, tuberculosis death rates in the city of Rio de Janeiro topped 1,000 per 100,000 inhabitants. They hovered around 500 in 1900, approximately 400 in 1920, and more than 300 in 1940. By 1950 a definite decrease to 192.9 was apparent. A remarkable phenomenon was the sharp plunge from 1860 to 1900 despite the lack of any organized program or specific measures. Even when yellow fever, smallpox, or plague epidemics prevailed, tuberculosis pre-empted throughout that period the first place in mortality statistics. It lost its pre-eminence only in 1950, when it was outstripped by cardiovascular disease. Pulmonary tuberculosis had a death rate of 184, and nonpulmonary tuberculosis of 8 in 1950. The rate for males was 235 and for females 159; among whites, 135; and among non whites, 331.9. By ages, the highest incidence (299) was in the 60- to 69-year-old group; the 50- to 59-year (284), the 20- to 29-year (283), and the 30- to 39-year-old (268) groups coming next. This is in contrast to 1940, when the 20 to 29, 30 to 39, and 40 to 49 age groups ranked highest. The highest rates now are in the most crowded, unsanitary, low-income districts. Antituberculous equipment in the city includes approximately 5,000 beds in sanatoriums and hospitals, 2 dispensaries, 26 ambulatories, and 9 mass survey units. BCG vaccination is being increasingly emphasized as an essential feature of the work performed by survey units.—*Evolução secular da mortalidade da tuberculose no Distrito Federal, A. F. Rodrigues de Albuquerque & B. de A. Rodrigues, Rev. brasil. tubérc., November–December, 1952, 20: 725.*—(*A. A. Moll*)

Fate of Patients with Pleurisy.—A review of 50 cases of serofibrinous pleurisy in adults treated at the central tuberculosis dispensary in Lima confirms the mildness of the condition in its acute period. The long-term prognosis is not so favorable; 58 per cent of the patients developed after a six-month to two-year period new pulmonary or nonpulmonary manifestations, or both. Of the patients, 37 were fifteen to thirty years old at the onset of the pleurisy.—*El porvenir de los pleuríticos, O. G. Lengua Bustios, Rev. tubérc., Habana, January–June, 1952, 12: 153.*—(*A. A. Moll*)

Rehabilitation in Tuberculosis.—During 1928 to 1952, a post-sanatorium rehabilitation center near Paris admitted patients whose tuberculosis had been inactive roentgenologically and bacteriologically for at least six months. After one week of medical observation and studies including aptitude tests, rehabilitation by practical and theoretical courses was started for three hours daily; this was accompanied by five hours of rest daily. If the reaction to this trial regimen was satisfactory, the activity was increased to six hours with only one hour of rest. This regimen was maintained for six to eight months. During the last phase of rehabilitation, the patient worked eight hours daily; there were no rest hours, and the daily routine was approximately that of a normal working schedule, although strict medical supervision was maintained. At this time the Social Service attempted to secure suitable employment for the patient after his discharge. Training was given in lathe work, milling, electromechanics, accounting, industrial design, poultry keeping, and some other branches of farming. Of the 416 patients discharged from the Center during the period of observation, 68 per cent are working and have resumed their place in the community; 55 per cent practice the trade which was learned at the Center. Employers usually do not discriminate against former patients who are willing to accept a forty-eight- to fifty-four-hour week. During residence at the Center, 43 patients (10 per cent) had relapses; 15 relapses during the first three months were probably due to poor selection for referral to the Center by the sanatorium physicians. After discharge from the Center, there were only 18 relapses, most of them dur-

ing the first year. The relapses seemed to be correlated with the preceding treatment and the degree of stability rather than with the strenuousness of the work; no statistical difference was found in the relapse rate of gardeners, factory workers, or accountants.—*Trois ans de fonctionnement d'un centre de réadaptation professionelle, Fichez & Datasta, Rev. de la tuberc., 1952, 16: 1129.*—(*V. Leites*)

Rehabilitation After Thoracic Surgery.—Postoperative rehabilitation by physical therapy is an integral part of the care of patients who undergo thoracic surgery. The results obtained by diligent corrective exercises have prevented, to a varying degree, scoliosis, "freezing" of the shoulder, matting, and atrophy. Comparative study of rehabilitated and nonrehabilitated cases emphasizes the advantages of these preventive and remedial measures. To aid in the education program, a booklet illustrating and describing the exercises has been prepared for patients. The effects of 18 exercises in 287 patients undergoing surgery have shown that the rehabilitative routine must be carried out for many months and, occasionally, for years.—*Rehabilitation in thoracic surgery, A. Haas, H. A. Rusk, & W. N. Goodman, J. Thoracic Surg., September, 1952, 24: 304.*—(*R. E. MacQuigg*)

High Tolerance to BCG.—A tuberculin-negative person was injected intramuscularly by mistake with 100 mg. of an eight-day BCG vaccine intended for oral use. An abscess developed at the inoculation site which ulcerated after twenty-five days. Roentgenograms two months later disclosed nothing abnormal; intradermal tuberculin tests up to 1:10 dilution were also negative. Two years and eight months after the injection, the roentgenographic findings, blood sedimentation rate, and blood picture were normal. An intradermal tuberculin reaction was positive at 1:100,000 dilution. Gastric lavage was negative. This seems to be the largest single dose of BCG ever injected intramuscularly. It was 2,500 times as large as the usual subcutaneous dose and 1,000 times as large as the average intradermal dose.—*Erro de administração do BCG (A propósito de uma injeção intramuscular de 100 mg. de BCG), A. C. de Moraes Passos & J. Soares Martins, Clin. tisiol., May-June, 1952, 7: 295.*—(*A. A. Moll*)

BCG Vaccination of Newborn.—Considerable skepticism relative to the value of BCG vaccination still exists. Special protective measures are indicated for a baby born of a mother who has been diagnosed as having active tuberculosis during pregnancy. A follow-up report of the first 100 newborn infants given BCG vaccination since 1950 reveals that in no instance did active tuberculosis develop. In 2 cases in which tuberculosis was suspected, the diagnosis was eventually not confirmed. There were no complications due to the vaccination itself.—*BCG—Schutzimpfung bei Neugeborenen von tuberkulosen Muttern, H. Jentgens, Tuberkulosearzt, 1952, No. 11: 667.*—(*E. Danner*)

Tuberculin Positivity Among Lepers' Children.—At a home for lepers' children, 100 inmates were tested with tuberculin and 75 of the group failed to react. Only 3, all negative, showed roentgenographically any pulmonary abnormalities. The entire anallergic group was immediately vaccinated with BCG.—*Cadastro tuberculino torácico em filhos de hansenianos no Educandário Vista Alegre, A. J. Abunahman & J. B. Risi, Clin. tisiol., May-June, 1952, 7: 321.*—(*A. A. Moll*)

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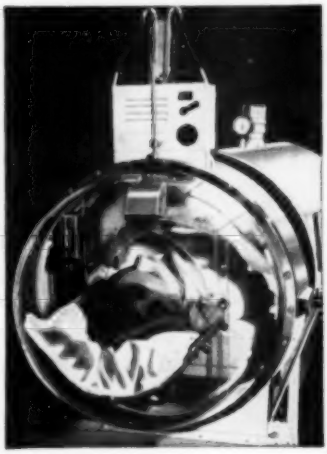

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1. Hobby, G. L., Lenert, T. F., Rivoire, Z. C., Donikian, M., and Pikula, D.: Am. Rev. Tubere. 67:808, 1953.

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